Emission of NH_3, N_2O and CO_2 following the application of differently treated digestates from biogas production

Von der
Fakultät Architektur, Bauingenieurwesen und Umweltwissenschaften
der Technischen Universität Carolo-Wilhelmina
zu Braunschweig

zur Erlangung des Grades einer
Doktorin der Naturwissenschaften (Dr. rer. nat.)
genehmigte

Dissertation

von
Ulrike Wolf
geboren am 19.10.1984
aus Bayreuth

Eingereicht am: 08. Mai 2014
Disputation am: 31. Juli 2014

Berichterstatter: Prof. Dr. R. Nieder, Technische Universität Braunschweig
Prof. Dr. H. Flessa, Georg August Universität Göttingen

2014

Table of Contents

List of tables

List of figures

List of abbreviations

AD	Anaerobic digestion
ANOVA	Analysis of variance
C	Carbon
CH_4	Methane
CO_2	Carbon dioxide
C_{org}	Organic carbon
LF	Liquid fraction
N	Nitrogen
N_2	Molecular nitrogen
N_2O	Nitrous oxide
NH_3	Ammonia
NH_4^+	Ammonium
NI	Nitrification inhibitor
N_{min}	Mineral nitrogen
NO_3^-	Nitrate
N_t	Total nitrogen
O_2	Oxygen
SF	Solid fraction
TAN	Total ammoniacal nitrogen
WFPS	Water filled pore space

Summary

Biogas is gaining growing importance as renewable energy source in Germany and Europe and the amounts of substrate residues from biogas production, called digestates, are concomitantly increasing. The composition and properties of digestates largely depend on its feedstock which is currently dominated by the use of dedicated energy crops, namely maize in Germany. The use of digestates as nitrogen (N) fertilizer can help closing nutrient cycles in crop production and contributing to an efficient resource management through partial substitution of mineral fertilizers. In addition, fertilization with digestates can contribute to soil carbon (C) sequestration and to a sustainable management of soil organic C in rotations with biogas crops. However, there are also potential negative effects of the increasing digestate application to agricultural soils. The use of digestates in crop production can induce considerable emissions of ammonia (NH_3), in addition, there might be the risk of increased emissions of the greenhouse gas nitrous oxide (N_2O). Different technical options such as mechanical separation, chemical additives, and different application techniques are discussed as potential measures to reduce emissions of NH_3 and N_2O from digestate application. However, field studies on mitigation of NH_3 and N_2O emission from application with different types of digestates under common agricultural conditions are scarce. The value of different digestates for soil C sequestration is also uncertain because there are only a few studies on mineralization dynamics and stability of digestate carbon in well aerated agricultural soils.

The objectives of this thesis were therefore to: i) quantify and compare NH_3 emissions after field application of different animal manures and digestates (from animal manures and digested maize) and to determine the effect of mechanical substrate separation on NH_3 emission, ii) determine the effect of different fertilizer types (i.e. calcium nitrate, digestate from maize, digestate from maize with a nitrification inhibitor) on NH_3 and N_2O emission from a maize field and to evaluate the total greenhouse gas budget of these fertilization systems and iii) analyze the processes of denitrification and C mineralization following application of ^{13}C and ^{15}N labeled digestate from maize simulating different application techniques in the laboratory.

In a field experiment performed in northern Italy, NH_3 emissions were measured following the application of different animal manures (dairy cattle and pig slurry) and digestates (from pig and dairy cattle slurries and digested maize) as well as their separated liquid and solid fractions. The substrates were applied to a maize field in spring and autumn. Compared to the non-digested substrates, digestion increased NH_3 emissions. Mechanical separation in total had no influence on the emissions. Between 2% and 33% of the applied NH_4^+-N was lost via NH_3 volatilization and especially the solid fraction of the separated substrates showed remarkably higher NH_3 losses compared to the non-separated substrates. Particularly for maize digestates, a trend for highest initial NH_3 volatilization rates was observed. The results underline the importance of immediate and complete incorporation of digestates into the soil to minimize NH_3 losses.

In a second experiment, the effect of a nitrification inhibitor (NI) on NH_3 and N_2O emissions was investigated in an annual field study. Different fertilizer types (i.e. calcium nitrate, digestate from maize, digestate from maize with a NI) were applied to an arable soil cropped with maize in central Germany. In the two periods following the application of maize digestate, the NI temporarily reduced N_2O emissions by 37% and 62%, but there was no effect on the annual scale. Further, the NI had no effect on NH_3 emission which in total accounted for nearly 30% of the applied digestate-NH_4^+-N. For the treatments with digestate application, the total fertilization related greenhouse gas emission was

dominated by the indirect N_2O emissions which were calculated from the measured NH_3 losses. In the treatment fertilized with $Ca(NO_3)_2$, which was established as a reference, the emissions from fertilizer production were the most important source of greenhouse gas emission since no NH_3 losses occurred and N_2O emissions were on the same low level as for the digestate treatments.
The effects of digestate application technique on the dynamics of C mineralization and denitrification were analyzed in a laboratory experiment. Fresh and digested maize material, both enriched in ^{13}C and ^{15}N, was incubated with soil under controlled conditions to trace the pathways of emitted CO_2, N_2O and N_2. The labeling approach enabled the differentiation of digestate C pools with different activity: a less processed fraction (isotopically labeled) and a longer processed fraction (without labeling). Mineralization dynamics of both C pools and denitrification (emission of N_2O and N_2) were quantified simulating different application techniques (i.e. surface application, homogenous incorporation and slit injection). The application technique had only a minor influence on mineralization of digestate C. The results reveal that digestates contained a very labile organic C pool (approximately 20% of the total organic digestate C) which was rapidly mineralized within the first two weeks after application. The less processed C fraction of the digestate showed even higher specific C mineralization rates than incorporated fresh maize biomass. Emission of N_2O was lowest for surface applied digestate and it was similar for all of the other tested incorporation techniques. Total denitrification (N_2O+N_2) and emission of N_2O were higher following application of fresh maize biomass than digested maize. The results suggest that denitrification was primarily restricted by C availability which promotes the formation of microzones with oxygen deficiency. Moreover it is indicated that the product ratio of denitrification ($N_2O/[N_2O+N_2]$) was influenced mainly by differences in soil nitrate availability.

The results show the great importance of biogas digestates as a new and growing source of NH_3 emission. Effective NH_3 mitigation measures are urgently required to benefit from the fertilizer value of digestates and to avoid this ecologically harmful loss of reactive N. Composition and properties of digestates can vary considerably depending on the digested substrates and the digestion process. This hampers the generalization of results on N and C dynamics and it suggests that specific evaluations are necessary for digestates of different origin. Digestates from maize contain a fraction of easily available organic C which affects not only the value of digestate for C sequestration in soils but it also triggers gaseous N losses by denitrification.

Zusammenfassung

Die zunehmende Bedeutung von Biogas als erneuerbare Energiequelle in Deutschland und Europa führt zu steigenden Mengen an Gärresten aus der Biogasproduktion. Die stoffliche Zusammensetzung sowie die Eigenschaften dieser Gärreste werden im Wesentlichen von ihren Ausgangssubstraten bestimmt. Gegenwärtig kommen hierfür in Deutschland überwiegend nachwachsende Rohstoffe (insbesondere Mais), die alleine oder mit anderen Zusätzen wie z.B. Gülle kofermentiert werden, zum Einsatz. Durch die Verwendung von Gärresten als Stickstoffdünger im Pflanzenbau können Nährstoffkreisläufe geschlossen und der Einsatz von Mineraldüngern verringert werden. Die Ausbringung von Gärresten kann zur Speicherung von Kohlenstoff (C) im Boden führen und zu einer nachhaltigen Steuerung des Boden-C-Kreislaufs in Fruchtfolgen mit Energiepflanzen

beitragen. Als negative Umweltwirkungen der zunehmenden Ausbringung von Gärresten können jedoch erhöhte Emissionen von Ammoniak (NH_3) und/oder von klimarelevantem Lachgas (N_2O) auftreten. Als Möglichkeiten zur Verringerung dieser Emissionen werden beispielsweise die mechanische Separierung des Substrats, der Einsatz chemischer Zusätze und verschiedene Ausbringungstechniken diskutiert. Allerdings wurden diese Strategien zur Minderung von NH_3- und N_2O-Emissionen nach der Ausbringung verschiedener Gärreste unter landwirtschaftlichen Praxisbedingungen bislang kaum in Feldstudien untersucht. Unklarheit besteht außerdem über die Auswirkungen verschiedener Gärreste auf die C-Speicherung im Boden. Nur wenige Studien haben sich der Mineralisationsdynamik und der Stabilität des Gärrest-C in gut belüfteten Ackerböden gewidmet.

Die Ziele dieser Arbeit waren daher: i) die NH_3-Emissionen nach der Ausbringung von verschiedenen tierischen Güllen und Gärresten (aus tierischen Güllen oder vergorenem Mais) zu erfassen und den Einfluss von mechanischer Substratseparierung auf die NH_3-Emissionen zu untersuchen, ii) den Einfluss verschiedener Dünger (Kalksalpeter, Maisgärrest und Maisgärrest mit Nitrifikationshemmstoff) auf NH_3- und N_2O-Emissionen aus einer Maiskultur zu quantifizieren sowie die Treibhausgasbilanz dieser Düngestrategien zu ermitteln und iii) in einem Laborversuch mit verschiedenen Ausbringungstechniken den Verlauf der Denitrifikation und der C-Mineralisierung eines ^{13}C und ^{15}N markierten Maisgärrests zu untersuchen.

In einem Feldversuch in Norditalien wurden NH_3-Emissionen nach der Ausbringung verschiedener Güllen (Milchvieh, Schwein) und Gärreste (aus den tierischen Güllen, Mais) sowie deren separierter Flüssig- und Festphase ermittelt. Die Substrate wurden im Frühjahr und im Herbst in einer Maiskultur ausgebracht. Hierbei zeigte sich, dass die NH_3-Emissionen auf den Teilparzellen mit vergorenen Substraten erhöht waren. Die mechanische Aufbereitung hatte insgesamt keinen Einfluss auf die NH_3-Emissionen. Durch die NH_3-Emissionen wurden 2 bis 33% des ausgebrachten Ammoniumstickstoffs (NH_4^+-N) freigesetzt. Dabei zeigte die feste Phase gegenüber den nicht-separierten Substraten wesentlich erhöhte NH_3-Emissionen. Insbesondere die Maisgärreste hatten eine Tendenz für erhöhte Emissionsraten unmittelbar nach der Ausbringung. Die Ergebnisse heben die Bedeutung einer raschen und vollständigen Einarbeitung der Gärreste zur Minderung von NH_3 Verlusten besonders deutlich hervor.

In einem zweiten, einjährigen Feldversuch in Deutschland wurde der Einfluss eines Nitrifikationshemmstoffs (NH) auf die NH_3- und N_2O-Emissionen untersucht. Dafür wurden verschiedene Dünger (Kalksalpeter [$Ca(NO_3)_2$], Maisgärrest, Maisgärrest mit NH) in einer Maiskultur ausgebracht. Während sich N_2O-Emissionen durch den NH-Einsatz in den beiden Zeiträumen nach der Gärrestausbringung kurzzeitig um 37 und 62% verringerten, konnte kein Einfluss auf die jährlichen Gesamtemissionen festgestellt werden. Der NH hatte keine Auswirkung auf die NH_3-Emissionen. Insgesamt wurden fast 30% des NH_4-N im Gärrest als NH_3 freigesetzt. Aus den NH_3-Emissionen konnten die indirekten N_2O-Emissionen nach der Ausbringung berechnet werden. Bei den Varianten mit Gärrestausbringung zeigte sich, dass diese den größten Anteil der gesamten düngerbezogenen Treibhausgasemissionen hatten. In der Versuchsvariante mit $Ca(NO_3)_2$ waren die Emissionen aus dem applizierten Mineraldünger die Hauptquelle für Treibhausgasemissionen. In dieser Variante waren keine NH_3-Emissionen messbar, außerdem waren die N_2O-Emissionen auf demselben niedrigen Niveau wie in den Gärrestvarianten.

Der Einfluss verschiedener Gärrest-Ausbringungstechniken auf den Verlauf der C-Mineralisierung und auf die Denitrifikation (N_2O+N_2-Emissionen) wurde in einem Laborversuch untersucht. Um die Herkunft des gebildeten CO_2, N_2O und N_2 zu verfolgen, wurde frischer und vergorener Mais jeweils

mit ^{13}C und ^{15}N angereichert und unter kontrollierten Bedingungen mit Boden inkubiert. Der Markierungsansatz ermöglichte die Unterscheidung zwischen zwei C-Pools mit unterschiedlicher Aktivität innerhalb des Gärrests: ein weniger vergorener (isotopisch markiert) und ein stärker vergorener Pool (ohne Markierung). Folgende Techniken zur Gärrestausbringung wurden simuliert: oberflächliche Ausbringung, gleichmäßige Einarbeitung, Schlitzinjektion. Die Ausbringungstechnik hatte nur einen geringen Einfluss auf die Mineralisierung des Gärrest-C. Es zeigte sich, dass der Gärrest über einen sehr leicht verfügbaren organischen C-Pool (ca. 20% des gesamten Gärrest-C) verfügte, welcher innerhalb der ersten beiden Wochen nach Ausbringung mineralisiert wurde. Der weniger stark vergorene C-Anteil des Gärrests zeigte sogar höhere spezifische Mineralisationsraten als die eingearbeitete frische Mais-Biomasse. Bei oberflächlicher Gärrestausbringung lagen die geringsten N_2O-Emissionen vor. Es gab keine Unterschiede zu den anderen Ausbringungstechniken. Sowohl die gesamte Denitrifikation (N_2O+N_2) als auch die N_2O-Emissionen waren nach Ausbringung der unvergorenen Mais-Biomasse höher als bei vergorenem Substrat. Die Ergebnisse zeigen, dass die Denitrifikation vorwiegend durch die C-Verfügbarkeit gesteuert war, welche die Bildung von Mikrozonen mit Sauerstoffmangel fördert. Das Verhältnis der Endprodukte der Denitrifikation ($N_2O/[N_2O+N_2]$) wurde hauptsächlich durch Unterschiede in der Nitratverfügbarkeit gesteuert.

Die Ergebnisse verdeutlichen die starke Bedeutung der Biogasgärreste als neue und wachsende Quelle für NH_3 Emissionen. Um von ihrem Wert als Dünger profitieren zu können und gleichzeitig den Verlust von umweltschädlichem, reaktivem Stickstoff zu verringern, werden wirksame NH_3-Minderungsmaßnahmen für Gärreste dringend benötigt. Zusammensetzung und Eigenschaften von Gärresten können sich in Abhängigkeit von den eingesetzten Substraten und verschiedenen Prozessbedingungen sehr stark unterscheiden. Das erschwert die Verallgemeinerung von Ergebnissen zur N und C Dynamik und verdeutlicht, dass gezielte Untersuchungen für Gärreste aus unterschiedlichen Substraten nötig sind. Maisgärreste enthalten einen Anteil an leicht verfügbarem organischem C, der nicht nur die Güte des Gärrests hinsichtlich der C-Speicherung in Böden beeinflusst, sondern auch gasförmige N-Verluste durch Denitrifikation hervorruft.

1 General introduction

1.1 Biogas production and its residues

1.1.1 State of biogas production in Germany and Europe

Germany's current energy policy has scheduled to abolish the use of nuclear power plants for energy production by 2022. Moreover, it is seeking to lessen the energy generation from fossil resources to tackle climate change. The Federal Government committed to transform the energy supply system by increasing the proportion of renewable energies on the electricity supply to 30% by 2020 (BMU 2010). In 2012, energy derived from biomass (bioenergy) accounted for 30% of renewable energies which corresponds to a total contribution of nearly 7% to the national electricity supply. More than half of the German bioenergy (58%) is currently produced in biogas plants, which are constantly increasing in numbers, from 1,000 plants in the year 2000 to more than 7,500 plants in 2012 (FNR 2013). This large rise can be attributed to the passage of the first Renewable Energy Sources Act in 2000 which guaranteed stable financial subsidies for the feed-in of bioenergy. However, the installation pace of new biogas plants largely decreased from 2011 to 2012 and a further drop is expected for 2013 (EurObserv'er 2013). This is mainly attributed to an announced cut in feed-in tariffs by the national government which became effective on 1st January 2012.

The compliant binding target of the European Union (EU) is to increase the EU-wide renewable-based share of total gross final energy consumption to 20% by 2020 (Directive 2009/28/EC). In 2011, Biogas supplied 2.5% of EU-wide renewable-based electricity (BMU 2013), but proportions among the member states vary widely. Leading countries in the primary production of biogas in the EU are Germany (61%, 6.4 million tons of oil equivalents [Mtoe]), followed by the United Kingdom (1.8 Mtoe) and Italy (1.2 Mtoe). Afterwards there is a large gap to a group of nine countries being in the production range between 0.1 and 0.4 Mtoe, led by France (EurObserv'er 2013). These numbers illustrate that today, biogas in Europe is produced by only a few countries, of which Germany is by far the dominant player.

Biogas is either produced in landfill, sewage or farm biogas plants, whereby the latter dominate the European biogas production. In 2011, 28% of biogas was produced from landfill, 12% from sewage sludge and the remaining 60% from decentralized agricultural plants (EurObserv'er 2011). The extent to which these sources are developed varies from country to country. Farm biogas plants which are based on agricultural substrates, dominate in Germany, Italy and the Netherlands. In the UK and France however, landfill biogas is the main source (EurObserv'er 2013).

1.1.2 Substrates for biogas production

Biogas is considered a versatile energy source because the possibilities for use include production of electricity and heat energy as well as the manufacture of transport fuels. Moreover it is available throughout the year and storable. Biogas is produced during anaerobic digestion (AD), the process of decomposition of organic material by microbial organisms in an oxygen-free environment. All types of biomass can be used as input substrate (feedstock) as long as they contain carbohydrates, proteins, fats, cellulose and hemicellulose as main components (Weiland 2010). Suitable substrates for the digestion in agricultural biogas plants are energy crops, organic wastes and animal manures. Energy crops comprise e.g. maize, herbage, clover grass, Sudan grass or fodder beet (Amon et al. 2007).

In Germany, biogas produced in agricultural plants is used for cogeneration of heat and power on farms (Scheftelowitz 2013). Due to favorable legal frameworks, the input of renewable agricultural primary products continuously increased for several years from 2004 onwards. The proportion of different feedstock types on total substrate input according to a survey with biogas plant operators in 2013 is shown in Figure 1-1.

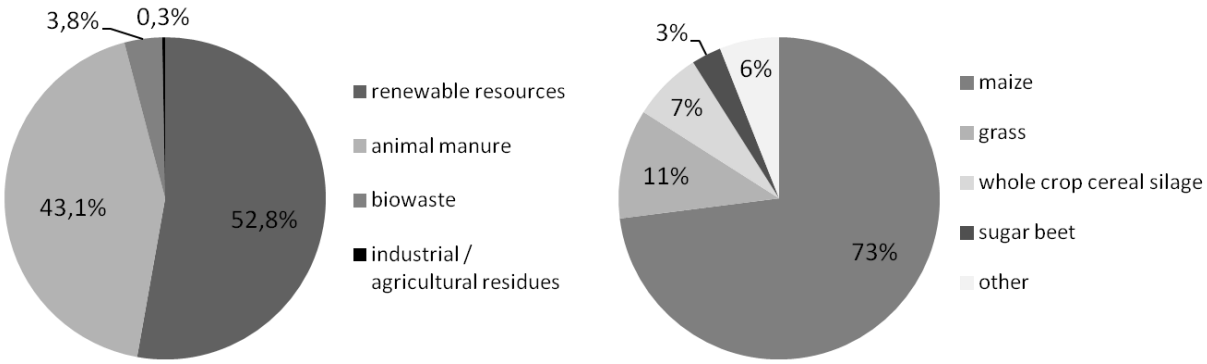

Figure 1-1: Proportions of input substrates (based on fresh matter) for agricultural biogas plants in Germany (left) and further differentiation within renewable resources (right) (Scheftelowitz 2013).

The addition of crops as co-substrates stabilizes anaerobic digesters and methane production can be strongly improved (Appels et al. 2011). Today, the majority of German biogas plants uses a mixture of manure and crops, 90-95% of all biogas plants use crops, several among them employ mono-digestion (Murphy et al. 2011).

The most important parameter for the selection of energy crops is their net energy yield per hectare. Maize is considered to have the highest yield potential of field crops grown in Europe. In 2013, nearly 13% of the total agricultural area in Germany was used for the cultivation of energy crops and 32% of the total maize growing area was cultivated with energy maize (FNR 2013). Especially for maize

cultivation, the increasing land consumption in several regions in Germany leads to a competition with production and the growing use as substrate for biogas production is seen critical (Herrmann 2013; Stinner & Rensberg 2011). In 2012 the additional subsidies for the use of energy crops in biogas plants were withdrawn by the German government. This may help to re-establish the preferential use of manure in biogas plants which is favorable because animal manure is available for free and easy to use with established digestion technologies. Moreover, digestion of animal manure avoids the volatilization of climate-damaging gases and odors during storage.

1.1.3 Digestate quality and use in agriculture

Anaerobic digestion is a complex biochemical process, which can be divided into four phases: hydrolysis, acidogenesis, acetogenesis/dehydrogenation and methanation (Weiland 2010). These phases are carried out by different groups of microorganisms, which decompose the complex organic compounds of the feedstock and produce methane (CH_4) during the final step. The final biogas is primarily composed of methane (50-70%) and carbon dioxide (20-45%) and contains small amounts of hydrogen sulfite and ammonia (NH_3).

The residual product of AD, called digestate, is commonly used as fertilizer. The quality of the digestate depends on the type of feedstock and the process technology used. In contrast to animal manure, plant-based substrates contain a bigger proportion of easily degradable organic material. Therefore, a higher degree of decomposition can be expected. During AD, about 20-95% of the feedstock organic matter is degraded, depending on the substrate composition (Möller & Müller 2012). The decomposition of organic dry matter leads to reduced contents of dry matter in the digestate, which vary between 2 and 13%. In the digester, complex organic nitrogen (N) compounds are mineralized to ammonium (NH_4^+), increasing the proportion of NH_4^+ on total N to 40-80% (Möller & Müller 2012). This represents a mean increase in NH_4^+ compared to the input material of around one third, highest increases were measured for the digestion of energy crops (Gemmeke et al. 2009). Digestates further have decreased total and organic carbon (C) contents, reduced biological oxygen (O_2) demands, elevated pH values (> 7.5), smaller carbon to nitrogen ratios (C/N ratio) and reduced viscosities compared to the input substrate (Asmus et al. 1988).

Albeit the chemical composition of the input material is largely changed during digestion, there is generally no alteration in total N, potassium (K) and phosphorus (P) (Field et al. 1984; Kirchmann & Witter 1992). Therefore, the application of digestate as a fertilizer to arable land ensures that crops receive most of the essential nutrients required for their growth (Arthurson 2009). Compared to feedstock, the lowered C/N ratio of the digestates results in decreased N immobilization which improves the short-term N-fertilization effect (Gutser et al. 2005). In the year of application,

digestates provide plant-available N according to their NH_4-N content plus a small part of the organic N fractions (10-20%) (Möller & Müller 2012). Repeated digestate application leads to accumulation of organic N in soil and contributes to enhanced long-term N release (Fouda et al. 2013).

Since nitrogen prevails in the form of NH_4^+, which is soluble and can be easily absorbed by plant roots, digestates are an efficient N fertilizer for cropping systems (Möller & Stinner 2009). Moreover, fertilization with digested cattle and pig slurries can result in higher yields than the application of commercial mineral fertilizers (Chantigny et al. 2008; Kocar 2008). Despite those reported beneficial effects of digestate use in cropping systems, it remains unclear whether the results are applicable to all types of digestates generated from a wide range of organic feedstock.

Besides N, phosphorus (P) is another essential nutrient for plant growth. The natural supply of P in most soils is low and for intensive cropping systems, P addition is required. However, repeated inputs of fertilizer and manure P in excess of crop requirements have led to a build-up of soil P levels, which are of environmental concern, particularly in areas of intensive crop and livestock production (Sharpley et al. 1994). Agricultural runoff and soil erosion from arable land leads to P input in freshwater lakes, streams and reservoirs and excessive concentrations of P is the most common cause of eutrophication in surface waters (Correll 1998). When digestates are applied to arable soil, thorough consideration of residual P levels is required to avoid surpluses with negative environmental impact. In areas with limited soil P contents, digestates can help to replace mineral P fertilizers, but mineral P supplementation is often necessary to avoid nutrient deficits (Svensson et al. 2004). The P contents of energy crops and animal manures vary in a wide range, so co-digestion may result in more balanced P contents in the digestates. Nevertheless, a sound P management system requires the calculation of application rates on soil vulnerability to P loss in runoff (Sharpley et al. 1994) and essentially needs substrate analysis before application.

The use of digestates can help to efficiently close agricultural nutrient and energy cycles. Anaerobic digestion prevents greenhouse gas emissions during the storage of animal manure, moreover, production-related greenhouse gas emissions can be avoided due to the substitution of inorganic fertilizers (Arthurson 2009). Nevertheless, the application of digestates can induce emission of climate-damaging gases, like NH_3 and nitrous oxide (N_2O), which reduce the fertilizer value and induce further environmental pollution.

1.2 Turnover of nitrogen and carbon after digestate application

1.2.1 N_2O emission

Besides methane and carbon dioxide, nitrous oxide (N_2O) is the third most important greenhouse gas and the importance of its contribution to the global climate change induced by human activity is well known (Ciais et al. 2013). Nitrous oxide contributes with 6% to the anthropogenic greenhouse effect (Forster et al. 2007), and is currently the most important contributor to the depletion of stratospheric ozone (Ravishankara et al. 2009). Nitrous oxide has an atmospheric lifetime of almost 120 years and related to the 100-year horizon, it is has a global warming potential of nearly 300, i.e. it is 300 times as potent as CO_2 as a global warming agent. The atmospheric concentration of N_2O is currently 20% higher than pre-industrial levels (324 ppb in 2011) and increased at a rate of 0.73 ppb yr^{-1} over the last three decades (Ciais et al. 2013). Anthropogenic sources were responsible for up to 39% of all global N_2O emissions in 2011, with an estimated total sum of 6.9 Tg N_2O-N yr^{-1}. Thereof, 4.1 Tg N_2O-N yr^{-1} are attributed to agriculture (Ciais et al. 2013). Anthropogenic emissions steadily increased during the last 20 years and N fertilizer used in agriculture is now the main source of N_2O emissions. This increase is mainly attributed to agricultural intensification comprising the enhanced use of synthetic fertilizers and manure applications which increase the production of N_2O in soils (Ciais et al. 2013).

Processes of N_2O formation in soils

In soil, different processes lead to the emission of N_2O as intermediate or end product during microbial transformation. Principal sources of N_2O are nitrification and denitrification processes (Bremner & Blackmer 1978), which are key transformation processes within the natural N cycle (Ussiri & Lal 2013) and contribute nearly 90% of the N_2O emissions from agricultural systems (Smith et al. 1997). Autotrophic nitrification and denitrification are considered as major N_2O forming processes (Figure 1-2), whereas nitrifier denitrification, heterotrophic nitrification and co-denitrification are supposed to be of minor importance for N_2O production in soil (Ussiri & Lal 2013).
Nitrification is the biological oxidation of NH_3 or NH_4^+ to nitrite (NO_2^-) and ultimately to nitrate (NO_3^-) under aerobic conditions (Figure 1-2), carried out primarily by autotrophic bacteria (autotrophic nitrification). *Nitrosomonas* oxidize NH_4^+ to NO_2^- with hydroxylamine (NH_2OH) as an intermediate and N_2O is produced during the further decomposition of NH_2OH. The oxidation of NO_2^- to NO_3^- is carried out by *Nitrobacter*. The rate of NH_4^+ oxidation is mainly regulated by NH_4^+ availability and the O_2 level in the soil; moreover, soil texture, soil moisture content and aeration, temperature, as well as pH influence nitrification (Sahrawat 2008; Subbarao et al. 2006).

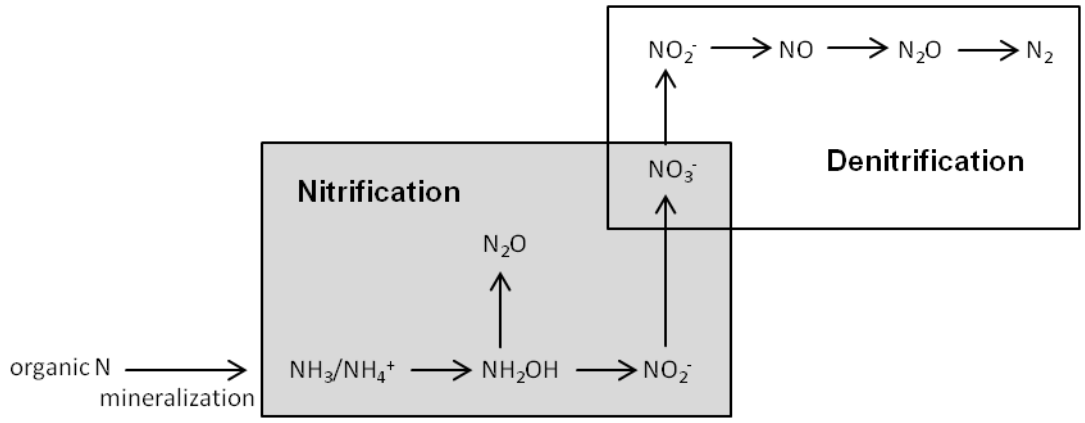

Figure 1-2: Major pathways of N_2O production in soil (modified after Wrage et al. (2001))

During denitrification, NO_3^- is reduced to NO_2^-, nitric oxide (NO), N_2O or N_2 under anaerobic conditions (Figure 1-2). This reduction can be performed by numerous and widespread bacteria (e.g. *Pseudomonas*, *Bacillus*) (Knowles 1982). The activity of denitrifying bacteria is controlled by NO_3^- and O_2 availability (mainly through soil water content or water filled pore space), soil pH and temperature (Baggs & Philippot 2010). Besides, C availability affects denitrification because it provides the electron donor for NO_3^- reduction (Bremner 1997). Though, C availability may not be a limiting factor under anaerobic conditions since O_2-stressed cells excrete more available C and therefore C limitation to denitrification is rare (Ussiri & Lal 2013). Otherwise available C influences soil microbial respiration and can lead to anaerobic microsites created by the decay of organic material – which in turn can promote denitrification. As a result, denitrification can also occur in aerated soils, where the formation of (temporarily) O_2-restricted microsites is promoted through enhanced microbial respiration (Barton et al. 1999).

The denitrification N_2O/N_2 product ratio is variable and N_2O may also be the dominant end product (Ussiri & Lal 2013). Under anaerobic soil conditions, both the rate of denitrification and the $N_2O/(N_2O+N_2)$ ratio must be known to finally evaluate N_2O emissions from denitrification (Mosier et al. 1996). According to Smith (1990), soil structure and water content have a strong influence on the proportions of the two gases, because both factors affect the balance between diffusive escape as N_2O and its further reduction to N_2. Moreover, the availability of NO_3^- regulates the N_2O/N_2 ratio: high NO_3^- concentrations can inhibit the conversion of N_2O to N_2 (Blackmer & Bremner 1978).

N_2O emissions from fertilized soils

As described above, several factors influence the rates of nitrification and denitrification and thus also N_2O emission from soils. Besides other effects related to management practices, fertilizer N input strongly controls N_2O emission from agricultural systems. Ammonium and nitrate are both

substrates for nitrification and denitrification and the level of mineral nitrogen (NH_4^+ and NO_3^-) content in soil influences the rate of N_2O emission. Under the prerequisite that the substrates are the only process limiting parameters, NH_4^+ stimulates nitrification and NO_3^- increases both denitrification rate and the N_2O/N_2 ratio. The application of mineral fertilizers or animal manures has frequently been shown to increase N_2O emissions (Bouwman et al. 2002a; Eichner 1990; Flessa & Beese 2000; Jarecki et al. 2008; Rochette et al. 2004; Stehfest & Bouwman 2006). In comparison with inorganic fertilizers, the application of animal manures and slurries to soil generally leads to higher N_2O emissions (Thangarajan et al. 2013), especially when applied to soils with low C content (Pelster et al. 2012; Rochette et al. 2000; van Groenigen et al. 2004; Velthof et al. 2003). Unlike mineral N-fertilizers, application of organic fertilizers provides C and increases the content of NH_4^+ which can be quickly nitrified and subsequently denitrified. This can result in higher N_2O emission per applied unit N compared to mineral fertilizers (Dendooven et al. 1998; Kaiser & Ruser 2000).

Due to these findings, increased N_2O emission can also be expected after digestate application. However, total N_2O emission and emission dynamics may differ from those of animal manure since the digestates have undergone process of anaerobic decomposition in the digester. Studies on N_2O emission following the application of digestates to arable soils basically revealed similar effects as observed for animal manures, with generally enhanced N_2O emission compared to unfertilized soils or mineral fertilizer application. But it was frequently reported that anaerobic digestion can reduce the risk of N_2O emission or denitrification losses compared to undigested material (e.g. Chantigny et al. 2007; Clemens & Huschka 2001; Petersen 1999; Rubaek et al. 1996; Vallejo et al. 2006; Wulf et al. 2002b), which was attributed to less easily available organic carbon in the digestates (Petersen 1992).

However, most studies which investigated the effect of digestates on N_2O emission covered only a few weeks after application, annual or even long-term studies are scarce. To evaluate the impact of fertilizer type on N_2O emission, at least annual datasets are required since N_2O emissions can be severely impacted by climatic conditions, e.g. freeze-thaw cycles which were shown to account for up to 50 to 75% of the annual N_2O flux (Dörsch et al. 2004; Flessa & Beese 1995).

Additionally, in particular the effect of digestates derived from energy crops on N_2O emission has not been evaluated as extensively as that of other organic fertilizers. To better understand the effects of different digestates on N_2O emissions, investigations both under field conditions and controlled conditions are required. Field studies are essential to quantify N_2O emission from different environmental conditions on regional and global scales. Apart from that, experiments under controlled conditions, i.e. laboratory incubations, are needed for detailed studies on how digestates affect other factors regulating N_2O emission.

1.2.2 NH_3 emission

Ammonia is another important N compound emitted to the atmosphere from agricultural systems. The volatilization of nitrogen as NH_3 results from agricultural fertilizer applied to land and from manure management. The atmospheric lifetime of reduced N (NH_x = NH_3 + NH_4^+) varies between hours and days and leads to temporally and regionally varying atmospheric concentrations (Denman et al. 2007). In the atmosphere, NH_3 is transformed to other N-containing compounds through reaction with atmospheric nitric acid (HNO_3) or sulphuric acid (H_2SO_4) to form ammonium nitrate or ammonium sulphate aerosols which are then further transformed to particulate NH_4^+ (IPCC 2006). Basically, all emitted NH_3 is returned to the surface by deposition which causes soil acidification and eutrophication of natural ecosystems (Bouwman et al. 2002b). After deposition on soil and water relatively close to their source, these reactive N compounds can cause N_2O formation. These emissions, resulting from the re-deposited NH_3, are considered as indirect N_2O emissions. Due to the severe environmental impact of pollutants like NH_3, the EU has implemented fixed ceilings of national NH_3 emissions (Directive 2001/81/EC). Member States have to report their emission inventories to the European Environment Agency in order to monitor progress and verify compliance. Anthropogenic emission account for around 79% of total global NH_3 emissions to the atmosphere, and agriculture is responsible for 76% of these (Ciais et al. 2013). On a global scale, annual NH_3 emission from agricultural systems are in the range of 27-38 Tg NH_3-N, including the use of N fertilizer with 10-12 Tg yr^{-1} and livestock production with 16-27 Tg yr^{-1} (Beusen et al. 2008). In Europe, 80% of agricultural NH_3 emission originate from livestock production (Hutchings et al. 2013), which includes animal houses and storage systems, the spreading of animal manure and animal grazing (Asman et al. 1998). Greatest proportions of NH_3 emission from European livestock production arise from buildings housing livestock and following application of manures to land, each of which typically accounts for 30–40 % of total NH_3 emission from livestock production (Hutchings et al. 2013).

Formation of NH_3 and its volatilization

Ammonia (NH_3) is constantly formed in soils from biological degradation of organic material or from NH_4^+ supplied by synthetic or organic fertilizers. At normal atmospheric temperatures, NH_3 is a gas which reacts readily with protons, metals and acidic compounds to form ions, compounds and complexes of varying stability in aqueous solution or solid forms (Freney et al. 1983). Thereby, NH_3 is protected from volatilization. In solution, NH_3 is in equilibrium with NH_4^+ which depends on pH value. The percentages of NH_3 in solution at pH 6, 7, 8 and 9 are approximately 0.1, 1, 10 and 50 – thus, there is a far greater potential for NH_3 volatilization at higher pH values (Freney et al. 1983).

Ammonia volatilization occurs when NH_3 in solution is exposed to the atmosphere and is driven by the differences in NH_3 concentrations between the surface solution and the overlying air.

Agricultural NH_3 losses occur primarily from the surface of liquid ammoniacal solutions, which are found in association with animal slurries, urine, solid manure and digestates. As summarized by Hutchings et al. (2013), NH_3 emission from field-applied manures depend on:

- substrate properties, including viscosity, total ammoniacal nitrogen (TAN = NH_4^+ + NH_3) content, C content and pH;
- soil properties such as pH, cation exchange capacity, calcium content, water content, buffer capacity and porosity;
- meteorological conditions including precipitation, solar radiation, temperature, humidity and wind speed;
- method and rate of substrate application, including, for arable land, the time between application and incorporation, and method of incorporation;
- height and density of any crop present.

These factors regulate either directly or indirectly the amount of NH_3 emissions after field application. Figure 1-3 shows their influence on NH_3 emission as analyzed by Sommer and Hutchings (2001). Ammonia volatilization starts immediately after substrate application and up to 50% of total emission can occur within the first few hours (Misselbrook et al. 2002).

It is assumed that the higher pH and NH_4^+-N contents of digestates predominate over factors which could reduce NH_3 volatilization from digestate application (lower viscosity and dry matter content) and result in higher NH_3 losses compared to animal manures (Möller & Stinner 2009). However, it is hard to disentangle the relative importance of single effects due to the complex interactions of factors regulating NH_3 emission after field application (Figure 1-3). Not surprising, differing results about the effect of digestion on NH_3 emissions have been reported. Several studies found enhanced emissions from digestates compared to non-digested material (Gericke et al. 2011; Moitzi et al. 2007; Möller & Stinner 2009; Nyord et al. 2012; Sommer et al. 2006) whereas no clear effect of digestion on NH_3 emissions was measured as well (e.g. Chantigny et al. 2009; Clemens et al. 2006; Wulf et al. 2002a). Ammonia emission related TAN content is highly variable, resulting from different substrates and environmental conditions and especially application techniques. However, the generally large N loss through NH_3 volatilization considerably reduces the fertilizer value of the substrate.

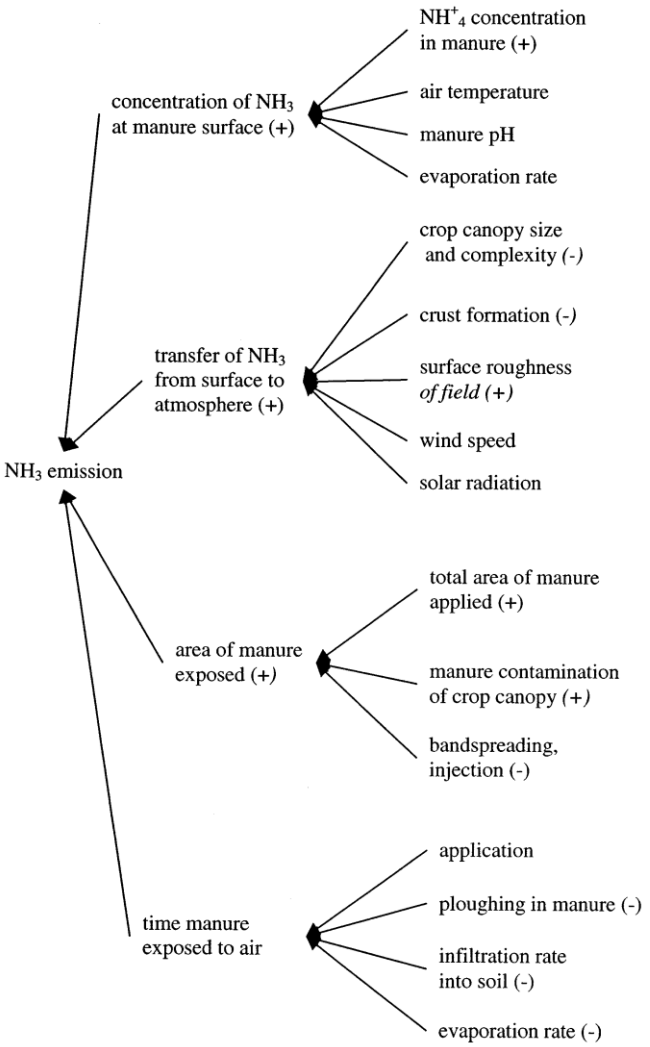

Figure 1-3: Factors affecting NH_3 volatilization following the field application of manures (Sommer & Hutchings 2001). Factors with a direct effect are shown in the first column and the factors with indirect effect in the second column. Some factors enhance NH_3 volatilization (+), others reduce NH_3 losses (-).

To date, no particular emission factors for digestates are available for the calculation of national emission inventories. Ammonia emission from digestates is calculated by using the emission factors for animal manure application although there is evidence that digestion increases NH_3 emission. Beyond that, there are first studies indicating that particularly digestates from energy crops may induce higher NH_3 emissions compared to digestates from animal manures (Ni et al. 2012; Pacholski et al. 2010). Thus, in particular with regard to the growing usage of plant-derived digestates, further investigations under field conditions are needed.

1.2.3 Carbon turnover

During anaerobic digestion, organic matter transformation is started in the digester resulting in the concentration of more recalcitrant molecules (e.g. lignin-like material, complex lipids, steroids) in the digestate (Kirchmann & Lundvall 1993; Marcato et al. 2009; Tambone et al. 2009). Since these molecules are considered as humus precursors (Lorenz et al. 2007), they play an important role in short-term soil organic matter (SOM) turnover after field application. With respect to their effect on soil fertility, digestates were shown to significantly increase both the microbial activity and the proportion of active soil microbial biomass (Kirchmann 1991; Odlare et al. 2008).

Soil organic matter is decreasing in arable soils under intensive production of most energy crops, and in particular intensive maize cropping consumes organic matter (OM) (Körschens 2004; Möller et al. 2011). Therefore amendments which increase the OM content are of special interest. The inputs of OM in soil play a central role regarding the productivity of arable land, as they provide nutrients during their decomposition and maintain soil fertility through OM turnover (Haynes & Naidu 1998).

When organic material is applied to soil with the purpose to increase soil properties and fertility, it must act as amendment. Materials are considered as amendments when their substrates contain organic fractions which contribute to SOM turnover, which in turn influences biological, chemical and physical soil characteristics (Lal 2001). In contrast, fertilizer properties describe the amount of nutrient elements contained in the organic material (Rowell et al. 2001). Animal manures were shown to contribute to increasing soil organic carbon stocks (e.g. Haynes & Naidu 1998), digestates, in contrast, are not yet comprehensively characterized regarding their amendment properties. The German C balance approach according to the German Cross Compliance commitments assumes digestate application to accumulate an equal amount of humus C as cattle slurry, but less than farmyard manure or compost (Körschens 2004). Opposed to that, Tambone et al. (2010) evaluated the degree of recalcitrance of digestate (digested municipal waste) as comparable to compost and much higher than of the undigested material (animal slurries mixed with crops or silage maize). The results were obtained from a laboratory study by analysis of the biological stability of the substrates. Several field or incubation studies with digestate application to soil showed that although the organic material has reached a higher degree of stability after digestion, around 30% of the added C were lost as CO_2 (e.g. Cayuela et al. 2010; de la Fuente et al. 2013; Terhoeven-Urselmans et al. 2009). However, digestates were more resistant to mineralization compared to undigested manures (Kirchmann & Lundvall 1993; Marcato et al. 2009) and according to modeling approaches, C accumulation in soil of up to 50% of the added digestate-C may be expected after one year (Cayuela et al. 2010). Nevertheless, particularly long term field studies are needed to evaluate effects on SOM turnover and stocks (Tambone et al. 2010).

Digestates from energy crops are usually returned to the cultivation area, which complies with the principle of closed nutrient cycles in agriculture. However, especially for intensive maize cropping systems, there are concerns that the reallocation of digestates cannot sufficiently compensate the SOC depletion (Ebertseder & Groß 2008; Möller et al. 2011) because the digestates are strongly depleted in organic C due to the digestion process. The amount necessary to achieve a balanced C budget in maize cropping systems strongly conflicts with nutrient balance limits, especially for P, set by the fertilizer ordinance (Herrmann 2013). Strategies to balance soil humus budgets on arable land therefore advise to leave high amounts of maize straw on the field instead of completely harvesting aboveground biomass (Möller et al. 2011), which is the usual practice for the use of energy crops. Nevertheless, to date, there are no experimental data whether the soil humus C effects of undigested maize straw are higher than the soil humus C effects of their digestates (Möller et al. 2011).

Digestates may function both as fertilizer and soil amendment, but their contribution to soil organic carbon turnover is has not yet been sufficiently evaluated. Additionally, the rate of OM decomposition during digestion depends on the input substrate, loading rate and retention time in the digester. Short retention times in combination with high loading rates will result in an uneconomic digestion process (maximum CH_4 yield was not achieved) (Menardo et al. 2011) and the digestate yields a low degree of OM stability. Analysis of C mineralization of single substrates may not adequately correspond to the variety among different digestate types and requires a comprehensive characterization.

1.3 Mitigation options for gas emissions after digestate application

1.3.1 Fertilization strategy

The use of an appropriate fertilization strategy enables farmers to maximize crop yield and reduce risks for N losses. Denitrification (N_2O and N_2), volatilization (NH_3) and leaching (NO_3^-) are the major pathways of N losses which cause a cascade of environmental and human health problems (Bouwman et al. 2010). Since highest NH_3 volatilization occurs directly after fertilizer application, strategies to mitigate NH_3 emission are mostly related to fertilizer application techniques (Chapter 1.3.3). In spite of the use of NH_4^+ conserving application techniques, several other requirements should be fulfilled to avoid N losses from applied digestate. In cropping systems, the N use efficiency by plants must be enhanced to retain N in the plant-soil-system, moreover site-specific management practices should be used (Bouwman et al. 2010). For a high N use efficiency, timing and matching of the N application with plant needs is essential, but sometimes difficult to achieve with organic fertilization. Adequate amounts of plant-available must be present during periods of high N demand, whereas minimal amounts of N should be present during periods of little N uptake (Möller et al. 2008). In maize production however, organic fertilizers (comprising digestates) are usually applied before or at sowing (Herrmann 2013) which bears the risk of N leaching during low N uptake in the early growth stage and especially in combination with precipitation events. The quick nitrification after digestate application and subsequent NO_3^- accumulation may induce higher N_2O losses through denitrification.

To obtain a balanced nutrient level, thorough consideration of the specific N demand of the crop and of the realistic site-specific yield expectations are essential (Gutser et al. 2005). Besides, consideration of the actual soil mineral N content is required to avoid nutrient surpluses which cannot be compensated by plant uptake. In particular the repeated application of animal manure or digestate leads to the accumulation of soil N (Gutser et al. 2005), which in turn can lead to an increased N release from the soil pool (Fouda et al. 2013). These residual effects should also be taken into account. Above all, exact information on the nutrient content of the digestate is crucial for the calculation of appropriate application rates and thus to avoid nutrient surpluses per se. Due to the large differences among the nutrient content of different digestates, overall estimates may be deficient.

1.3.2 Mechanical / chemical treatment of digestate

Digestate treatment technologies include mechanical (e.g. solid-liquid separation) or chemical (e.g. flocculation, acidification) options and, apart from other agronomic benefits, may help to reduce NH_3

volatilization upon field application. Physical solid-liquid separation is considered as first step of the treatment procedures and further treatment of the solid fraction (e.g. composting, drying) or liquid fraction of digestates (e.g. dilution, filtration, chemical additions) may follow (Hjorth et al. 2010; Möller & Müller 2012). Solid-liquid separation can be carried out with settling tanks, centrifuges, fabric belts/screens or screw-press separators, which are most commonly used (Hjorth et al. 2010; Moller et al. 2000). The separation results in a solid fraction (SF) with a high dry matter content and a liquid fraction (LF) with a low dry matter content. For digestates from energy crops with a high proportion of silage maize, the SF often comprises around 20 to 25% of the total digestate fresh matter (Möller & Müller 2012). After separation, the dissolved NH_4^+-N remains in the LF whereas the organic N is merely transferred to the SF (Moller et al. 2002). However, the total N concentration in the SF is still rather high and may induce N losses during handling and application (Möller & Müller 2012). The composition of the separated fractions can vary greatly, depending e.g. on separation efficiency (Hjorth et al. 2010; Moller et al. 2000; Moller et al. 2002).

Separation efficiency can be improved by chemical treatments such as the addition of flocculants which remove suspended particles (Hjorth et al. 2010). Flocculation is induced by the addition of polymers (e.g. polyacrylamide) and was approved as efficient in removing solids from the liquid fraction (e.g. Christensen et al. 2009; Rico et al. 2012). Since polyacrylamides can be toxic, treated substrate should not be reallocated on cultivated fields (Hjorth et al. 2010).

Ammonia emissions increase with increasing dry matter content of the substrate (Sommer & Olesen 1991) because the solids on the surface impede infiltration (Dosch & Gutser 1996). A lower dry matter content and thus rapid substrate infiltration is therefore supposed to reduce NH_3 losses (Sommer et al. 2004). Indeed, field application of the liquid fraction of pig manure (Chantigny et al. 2007; Chantigny et al. 2009) or digested pig slurry (Nyord et al. 2012) resulted in a 25% reduction of NH_3 emissions compared to the non-separated substrate. But otherwise, the solid fraction was identified as main source of NH_3 loss both during storage and after field application of separated slurry (Dinuccio et al. 2012). This should also be taken into account for the assessment of the total NH_3 mitigation potential of liquid-solid separation.

Acidification was shown to largely reduce NH_3 emissions from animal manure, since NH_3 volatilization is pH dependent and decreases with acidity. Ammonia emissions were reduced by 67 to 95% through the addition of nitric or sulfuric acid (Frost et al. 1990; Kai et al. 2008; Pain et al. 1990c; Stevens et al. 1989; Stevens et al. 1992). Despite these strong emission reductions, the use of acidification on the farm level is minimal for practical reasons (e.g. on farm handling with strong acids, manure foaming) (VanderZaag et al. 2011).

Technical treatment options can thus help to preserve the N in the digestate by reducing NH_3 volatilization. However, higher N contents in the substrate may in turn increase N_2O emissions after land application.

A promising option to reduce N_2O emission from added fertilizers is the addition of nitrification inhibitors (NIs). Nitrification inhibitors retard the bacterial oxidation of NH_4^+ to NO_2^- and thus can help to increase the efficiency of fertilizer N use by reducing NO_3^- leaching losses as well as nitrification losses as N_2O (Prasad & Power 1995). Nitrification inhibitors for the agricultural use are synthetic chemical compounds, available in liquid or solid form for the use with mineral fertilizers or animal manure. The persistence and efficiency of NIs depends on a number of environmental factors, such as soil temperature, soil pH, soil texture or soil organic matter content (Subbarao et al. 2006). Regarding their agronomic effect, there is no consistent evidence, that NIs increase N use efficiency (Robertson & Vitousek 2009). But NIs were shown to significantly reduce N_2O emission after application of synthetic fertilizers or manure in many field studies (Akiyama et al. 2010) and are considered as useful mitigation option for N_2O emission (Smith et al. 2007). Although the meta-analysis of Akiyama et al. (2010) assessed a mean reduction of N_2O emission by 38%, the size of the effect varied largely depending on type of NI, type of applied fertilizer, or land use type. Apart from that, also the duration of N_2O emission measurement after application of the NI can influence the evaluation of its efficiency. The application of NIs may delay N_2O emission patterns but annual studies on after NI application are scarce. Moreover, though NIs are the most widely tested mitigation option for N_2O emission from agricultural soils (Akiyama et al. 2010), no investigations on the effectiveness of NIs with digestate application are available so far.

1.3.3 Application technique

The reduction of the direct N loss via NH_3 volatilization from field-applied manure (and digestates) can be achieved relatively cost-efficient by selection of the appropriate application technique (Huijsmans et al. 2003). According to Misselbrook et al. (2002), there are four main types of slurry application systems in use on farms in Europe: broadcast spreading, band spreading (with trailing hoses), trailing shoe applicator and injection (5 to 15 cm depth). The common application method for slurry is surface broadcast spreading by splash plate, which is rapid and inexpensive (Webb et al. 2010) but is known to induce highest NH_3 losses (Huijsmans et al. 2003). The application technique substantially affects NH_3 volatilization because NH_3 volatilization can be reduced by minimizing exposure of manure surface to air and by increasing the contact of manure with soil (Sommer & Hutchings 2001). Thus, substrate incorporation and injection were most effective in reducing NH_3 emissions by up to 95% compared to surface spreading in several studies across Europe (Huijsmans

et al. 2003; Malgeryd 1998; Misselbrook et al. 2002; Smith et al. 2000; Thompson et al. 1987). The effectiveness of NH_3 emission abatement is influenced by differences between the conditions under which the application techniques are used (ambient, soil and crop conditions, operational factors) (Misselbrook et al. 2002). Band spreading with trailing hoses also reduces NH_3 emissions compared to broadcast spreading, but is less effective compared to substrate incorporation or injection (Malgeryd 1998; Smith et al. 2000). For slurry incorporation, the plough is recommended as most effective tool (Webb et al. 2010), but also the use of a harrow following broadcast spreading was shown to efficiently reduce NH_3 emissions on arable land (by 60%), provided that manure and soil are thoroughly mixed (Malgeryd 1998). Highest reduction of NH_3 is achieved with immediate substrate incorporation after spreading and further delay reduces the effectiveness (Webb et al. 2010).

The use of low NH_3 emission application techniques may however have the adverse effect on N_2O emissions (Thompson et al. 1987). This can firstly be attributed to the fact that more N is preserved in the soil which can potentially be converted to N_2O (Velthof & Mosquera 2011). Secondly, application techniques like injection expose the manure to deeper and wetter soil which can increase N_2O losses from denitrification (Webb et al. 2010). Higher N_2O emissions were sometimes reported after injection of animal slurry (Flessa & Beese 2000; Velthof & Mosquera 2011; Wulf et al. 2002b), but not consistently (Sommer et al. 1996; Velthof et al. 1997). Moreover, there are indications, that the injection depth may also influence N_2O emission (Velthof et al. 2003). Although it is likely that some reduced-NH_3 application techniques increase emission of N_2O, consistent evidence is missing.

1.4 Objectives of the present study

A better understanding of N and C dynamics after digestate application is crucial for the sustainable use of digestates in agriculture. Digestates are frequently compared with animal manures, but their quality differs due to the technical digestion process. Moreover, digestate quality can vary considerably with respect to used substrate and there is little information about the effect of digestates from energy plants on NH_3 and N_2O emissions and C turnover in soil.

Therefore, the research objectives of this thesis were to:

i) quantify and compare NH_3 emissions after field application of different animal manures and digestates (from animal manures and digested maize) and to determine the effect of mechanical substrate separation on NH_3 emission
ii) determine the effect of different fertilizer types (i.e. calcium nitrate, digestate from maize, digestate from maize with a nitrification inhibitor) on NH_3 and N_2O emission from a maize field and to evaluate the importance of N_2O and NH_3 for the total greenhouse gas balance of these different fertilization systems
iii) assess the effects of anaerobic digestion of maize biomass and of different digestate application techniques on mineralization dynamics of digestate C and gaseous N_2O and N_2 losses

Objectives i) and ii) were studied in two field experiments under conditions of common agricultural practice, whereas objective iv) was addressed in a laboratory incubation under controlled conditions.

The field experiment for objective i) was located in Emilia-Romagna, Northern Italy. NH_3 emissions were measured during two seasons (spring and fall) following the application of pig and cattle slurry, digestates from pig and cattle slurry and digested maize. Moreover, the liquid fractions of separated cattle and maize substrates were applied in spring, the respective solid fractions in fall. Field management (i.e. type of application, amount of substrate and incorporation) followed common practice in the study area.

In the second experiment (objective ii), the effect of a nitrification inhibitor (NI) on NH_3 and N_2O emissions was investigated in an annual field study in central Germany. Different fertilizer types (i.e. calcium nitrate, digestate from maize, digestate from maize with a NI) were applied to an arable soil cropped with maize. Digestate was applied with trailing hoses (split application), NH_3 emissions were measured directly after application for several days. Measurement of N_2O emission covered one year.

For objective iii), fresh and digested maize material, both enriched in ^{13}C and ^{15}N, was incubated with soil under controlled conditions to trace the pathways of emitted CO_2, N_2O and N_2. The labeling approach enabled the differentiation of digestate C pools with different activity: a less processed fraction (isotopically labeled) and a longer processed fraction (without labeling). Mineralization dynamics of both C pools and denitrification (emission of N_2O and N_2) were quantified simulating the following application techniques: surface application, homogenous incorporation, slit injection and closed slit injection.

2 Ammonia emissions following application of untreated, digested and separated slurries and digestates to an Italian maize field

2.1 Abstract

Increasing numbers of biogas plants in Europe produce increasing amounts of biogas residues. These digestates are considered valuable organic fertilizers. However, anaerobic digestion alters the chemical quality of the substrate (e.g. pH value or ammonium content), thereby potentially increasing the risk of ammonia (NH_3) volatilization after application as fertilizer. Thus, liquid manure from different animals (dairy cattle, pig) and different biogas digestates were compared regarding NH_3 emissions after application. Biogas digestates originated from the digestion of the two types of liquid manure and of maize residues. Additionally, the effect of applying liquid and solid fractions of cattle slurry and biogas residues was studied. In spring and fall 2010, 250 and 100 kg N ha^{-1}, respectively, of the substrates were applied on a maize field in northern Italy. In both seasons NH_3 emissions peaked immediately after application, reached maximum emission rates of 0.9 kg NH_3-N ha^{-1} h^{-1} and quickly decreased thereafter. In terms of NH_4^+-N applied, 5.8 to 13.3% and 1.7 to 32.8% were lost via NH_3 volatilization in spring and fall, respectively. Band application technique and crust formation after application in the spring season apparently lowered NH_3 emission rates despite warm air temperatures.Biogas digestion resulted in enhanced NH_3 volatilization compared to liquid manure both in spring and fall. Mechanical separation of the slurries had no influence on NH_3 emission in total. However, NH_3 volatilization related to NH_4^+-N content was remarkably higher for separated solids compared to non-separated substrates in fall. Our results demonstrate the need of emission factors adapted to specific substrate qualities for a reliable calculation of ammonia emissions from the warm temperate region.

2.2 Introduction

Processing animal slurries in biogas plants is a common practice, which reduces emissions of the greenhouse gas (GHG) methane (CH_4) from slurry storage and produces CH_4 as renewable bioenergy source (Holm-Nielsen et al. 2009). Biogas plants are not only fed with animal slurries and organic waste, but also with agricultural crops. Co-fermentation of animal slurries with energy crops increases the CH_4 yield, but GHG emissions during crop production reduce the mitigating effect on net GHG emissions. The digested residues (digestates) of biogas plants are valuable organic fertilizers. Anaerobic digestion in biogas plants changes the chemical composition, e.g. it increases ammonium (NH_4^+) content and pH value (Asmus et al. 1988). This results in an elevated risk of ammonia (NH_3) volatilization during storage and after application of biogas digestates to agricultural land. Thus, the increasing numbers of biogas plants in many European livestock-intensive countries are a growing new source of NH_3 emissions. Currently, agriculture emits around 90% of anthropogenic NH_3 emissions in Europe (EEA 2010). Ammonia emissions originate mainly from livestock effluents during animal housing and grazing, manure storage, and slurry application as

organic fertilizer (Asman et al. 1998; Sutton et al. 2011). Increasing NH_3 emissions from biogas digestates counteract the aims of the EU National Emissions Ceilings Directive from 2010, which sets upper limits for national NH_3 emissions. Ammonia losses reduce the value of slurries or digestates as nitrogen (N) fertilizer and they contribute to several environmental problems. As a precursor of NH_4^+ deposition, NH_3 emission contributes to soil acidification (Ferm 1998). Additionally, deposition of ammoniacal nitrogen (NH_3-N and NH_4^+-N) contributes to eutrophication and induces indirect emissions of the greenhouse gas nitrous oxide (N_2O) (Well & Butterbach-Bahl 2010).

Several factors are known to influence NH_3 volatilization following slurry application. Important controls of NH_3 emissions are (1) slurry properties such as dry matter content, viscosity, NH_4^+ content, and pH value (Braschkat et al. 1997), (2) weather and soil conditions, i.e. temperature, wind speed, soil texture and moisture (Sommer et al. 1991), and (3) slurry application techniques (Misselbrook et al. 2002).

Slurry injection or application with trail hoses instead of broadcast spreading with splash plates have been shown to successfully reduce NH_3 emissions (Lovanh et al. 2010; Misselbrook et al. 2002; Rubaek et al. 1996; Sommer et al. 1997; Webb et al. 2010). Additionally, mechanical separation of liquids and solids in slurry and biogas digestates has been discussed as an option for reducing NH_3 losses (Amon et al. 2006; Dosch & Gutser 1996), but the effect of this technique is not well known since field experiments with separated digestates are rare. Mechanical separation of slurries results in a nutrient-rich solid fraction and a liquid fraction with low dry matter content and a high concentration of dissolved nutrients. Separation efficiency varies widely depending on the separation technique and the physical and chemical composition of the substrates (Zhang & Westerman 1997). After separation, the solid fraction can be transported more cost-efficiently to regions with lower livestock densities and lower abundance of organic fertilizers. Therefore, this technique could facilitate a reduction of nutrient surplus in regions with intensive livestock farming. Faster infiltration into the soil due to a reduced dry matter content may also help to reduce NH_3 volatilization following application of the liquid fraction (Sommer & Hutchings 2001). However, separation may also increase the risk of NH_3 losses, especially during uncovered storage of solid fractions (Amon et al. 2006).

The objectives of our study were to (1) determine the effect of digestion and separation on chemical properties (e.g. mineral and total N content) of different animal and plant-derived slurries and digestates, to (2) quantify NH_3 emissions following the application and incorporation of different slurries and digestates, and to (3) investigate the effect of digestion and separation on NH_3 volatilization under two contrasting weather conditions and seasons. The study includes relevant types of slurries (dairy cattle, pig) and their digestates as well as a digestate derived solely from energy plants (maize) which represents a new type of fertilizer with growing importance. Investigated substrate treatments are anaerobic digestion and mechanical separation, both common

in agricultural practice. Practice-relevant amounts of substrate were applied and measurements were carried out under field conditions and in two different seasons of application.

2.3 Materials and methods

2.3.1 Experimental site and treatments

Investigations were carried out in Italy's main region of animal breeding, Emilia-Romagna. The experimental site was located approx. 15 km north-east of the city of Reggio Emilia on a field adjacent to the village of Gazzata (44° 42' 11" N, 10° 46' 03" E, San Martino in Rio municipality). The region enjoys a warm temperate fully humid climate with warm summers. Mean annual temperature in Modena, situated approx. 10 km east of the investigation area, is 13.8 °C and the annual precipitation is 843 mm. Soil texture of the Ap horizon (0-30 cm) of the field is 46% clay, 43% silt, and 11% sand (silty clay), which is typical for alluvial soils in the Po valley. Further soil properties are a $CaCO_3$ content of 120 g kg^{-1}, a pH (H_2O) of 8.1, a soil organic matter content of 25 g kg^{-1}, and a total nitrogen (N_t) content of 1.6 g kg^{-1}.

In April 2010, a field plot experiment was established to quantify NH_3 emission following the application of organic fertilizers. Tested fertilizers were dairy cattle and pig slurry, digestates from different substrates, and separated slurries and digestates (listed below). Application of the different substrates and subsequent measurements were carried out in May (spring application) to growing grain maize (*Zea mays, L.*) and in September 2010 after harvest (fall application). To establish conditions of common agricultural practice which are agriculturally and economically reasonable, the different fractions of the separated slurries were applied during different times of the year. Due to the different N availability, the liquid slurry fractions were applied to the growing crop (spring) whereas the solid fractions were applied after harvest (fall).

Consequently, the emissions from the two separated fractions of each substrate cannot be directly compared. Nevertheless, it was possible to assess the two application systems, i.e. application of non-separated slurry in spring and fall and on the other hand application of the liquid fraction in spring and the solid fraction in fall.

The following treatments were studied:

C: cattle slurry applied in spring and fall

C lf / sf: separated cattle slurry with liquid fraction (C lf) applied in spring, solid fraction (C sf) applied in fall

dC: digested cattle slurry applied in spring and fall

dC lf / sf: separated digested cattle slurry with liquid fraction (dC lf) applied in spring, solid fraction (dC sf) applied in fall

P: pig slurry applied in spring and fall

dP: digested pig slurry applied in spring and fall

dM: digested maize applied in spring and fall

dM lf / sf: separated digested maize with liquid fraction (dM lf) applied in spring, solid fraction (dM sf) applied in fall

control: soil without slurry application

Plots were 3.5 m x 5 m in size and included 6 rows of maize plants with a row width of 80 cm. Plots were separated lengthways by three rows of maize. In between the different treatment plots, a small metal board was installed to a depth of approximately 10 cm to avoid runoff of substrate in the case of heavy rainfall events. For each treatment one field plot was established. Plots were not replicated since spatial soil heterogeneity was low and variability of NH_3 emission of a given treatment was expected to depend more on small scale variability of application technique and substrate composition than on field heterogeneity. Thus, three replicated measurements of NH_3 volatilization were carried out randomly within the same field plot. The control plot (soil without application of organic fertilizer) was established downwind to capture any potential NH_3 drift from slurry-/digestate- treated plots, which allowed to quality-check the measured NH_3 flux differences between plots and to correct for NH_3 drift if necessary.

2.3.2 Slurry and digestate properties and application

Animal slurries were collected from pig fattening farms and a dairy cow production farm in the region of the investigation site. Biogas digestates of maize and cattle slurry were collected from farm-scale and pilot biogas plants. Cattle slurry and maize digestate were collected non-separated as well as separated into solid and liquid fraction by a sieve drum press (cattle slurry) or a screw press (maize digestate). As shown in Table 2-1, cattle slurries and digestates as well as maize digestate originated from the same farm in spring and fall, but pig substrates were derived from different farms in different seasons. Separation of pig slurry was not part of our study because of its low dry matter (DM) content.

The substrates were transported in big plastic tubs (50 l) and stored for one week at the field site with loosely covered lids until the day of application. On the days before application, representative substrate samples were taken upon careful stirring and mixing the substrates and analyzed for N content. The intended fertilization rate was 250 kg N ha^{-1} in spring and 100 kg N ha^{-1} in fall for all treatments, which are typical application rates in Emilia-Romagna. We checked the exact amount of N applied by a second sampling which was carried out at the day of substrate application. The

detailed results in Table 2-2 show that the applied N amount slightly differed from the calculated target values for single treatments.

Complete analysis of substrate composition was conducted with samples collected on the day of spreading. The pH of undiluted slurry was determined with a pH electrode (Crison instruments, Spain), slurry dry matter content was determined gravimetrically after oven-drying until constant weight at 105°C. Total nitrogen (N_t) was measured using the Kjeldahl method (Gerhardt analytical instruments, Germany), ammoniacal nitrogen via distillation and titration (Foss Tecator, Denmark), and total organic carbon (TOC) was determined after acidic digestion (Velp, Scientifica, Italy). The properties of the applied substrates in spring and fall are summarized in Table 2-1. The substrates were manually applied using watering pots or buckets. Similar to a regular trailing hose applicator, the substrates were placed in bands between the crop rows in spring and maize stubbles rows in fall. Spring application was carried out between 11 a.m. and 1 p.m., in fall substrates were applied between 9 and 11 a.m. Mechanical incorporation was conducted 21 hours after spreading in spring and in fall. This time lag is common practice in Emilia-Romagna. In spring, slurries were incorporated into soil to a depth of approximately 10 cm using a rotary harrow. In fall after maize grain harvest, amended soil and plant residues were plowed to a depth of 30 cm.

2.3.3 NH_3 volatilization measurements

Ammonia emission was determined with the Draeger-Tube-Method (DTM), an open dynamic-chamber method (Roelcke et al. 2002) that has been calibrated to account for ambient wind speed (Pacholski et al. 2006). It was successfully validated as a fully quantitative method in several studies on NH_3 emission from fertilized fields (Gericke et al. 2011; Quakernack et al. 2012). The results of DTM measurements are in close agreement with absolute NH_3 losses as it was calibrated on a high number of simultaneous measurements with a micrometeorological method (Pacholski et al. 2008), which is considered a highly precise methodology for quantifying NH_3 losses in the field. As the DTM measurement system is easy to move and handle and integrates over well defined small chamber areas, it allows measurements on relatively small field plots in a short time (about 5 minutes). The method requires no electricity or laboratory analyses, which makes it suitable for *in situ* conditions on real farms. The DTM is well suited to capture small scale variability within plots, e.g. different band widths and irregular applied amounts of the applied substrates.

The system consists of four tinplate cups (surface area 416 cm^2, height 15 cm, insertion depth 2 cm) with an air inlet and outlet in the lid. Via Teflon tubes, the four cups are connected and form one measurement unit. The cups were deployed on the field plots in such a way that they integrate representatively over fertilized and unfertilized field area (i.e. 2 cups were placed on the substrate amended area and two on the unfertilized field if half of the plot surface was covered with slurry

stripes). During the measurement, air is sucked by a hand pump (Type: *accuro*, stroke volume: 100 ± 5 cm³, Draeger Safety AG, Lübeck, Germany) which is connected to the air outlet tube. The air from the cups is conducted through an ammonia-sensitive Draeger indicator tube (Draeger Safety AG, Lübeck, Germany) where the concentration of ammonia gas in the air is immediately displayed. From the displayed NH_3 concentration, a NH_3 flux rate was calculated and adapted to ambient air velocity with the calibration approach of by Pacholski et al. (2006). Since the measurements were conducted in growing crop in spring and on bare soil in fall, we used the calculation recommended for winter conditions and growing crop, including ambient air velocity in 2 meters height. In the present study, meteorological data including the wind speed (2 m) were measured throughout the whole experimental period by a climate station nearby the field plots.

Ammonia measurements were started immediately after slurry application. Measurements were carried out in 3 replicates per treatment plot. Duration of NH_3 monitoring was 6 and 5 days in spring and fall, respectively. After this period, NH_3 fluxes were close to zero on all plots and thus it was ensured that the main period of emissions was captured.

Potential NH_3 drift between fertilized plots was considered by subtracting NH_3 emissions from the control plot (no substrate application) from NH_3 emissions measured from treatment plots. This correction is based on the assumption that there was no emission from unfertilized soil. The low NH_3 concentration measured at some sampling dates on the control plot were thus attributed to NH_3 drift from fertilized plots.

2.3.4 Statistical analyses

Analysis of variance (ANOVA) was used to compare the different treatments with regard to the composition of the applied substrates and the NH_3 emissions. If differences in the substrate composition between the treatments were evident ($p < 0.05$), the Holm-Sidak method was used as post-hoc test. NH_3 emissions and NH_3 emissions related to NH_4^+ applied were studied with a 3-way-ANOVA. Type of substrate, digestion and separation were taken as factors. Level of significance was set to $p < 0.05$. For the analysis, mean values for spring and fall application were calculated from the three measurements per plot. Data were square root-transformed to obtain normality of residuals and homogeneity of variances.

Cumulative NH_3 losses were calculated from linear interpolation between treatment mean values of two subsequent measurements. As data for NH_3 emissions during nighttime (between 9 p.m. and 5.30 a.m.) are lacking, linear interpolation for this period may slightly overestimate NH_3 emissions.

Since replicate measurements were carried out by placing the set of cups randomly onto a different spot of a plot during each measurement, it was not possible to calculate the standard deviation from cumulative replicate values. Therefore, a Monte-Carlo simulation was used for error propagation to

determine the standard deviation of cumulative NH_3 losses. This was done by drawing values from normal distributions with the calculated means and standard deviations for each time point and calculating a simulated cumulative value. The simulation was repeated often (n = 1e6) and the standard deviation of the simulated cumulative values is an estimate of the true standard deviation of the cumulative NH_3 losses.

2.4 Results

2.4.1 Composition of applied substrates

Chemical properties of the investigated slurries are shown in Table 2-1. In the spring season, digestion increased the pH value by 1.1 units (p = 0.03, Holm-Sidak test) and also in fall a trend for higher pH values by 0.9 units after digestion was observed (p = 0.07, Holm-Sidak test).

Table 2-1: Origin and properties of substrates applied in spring and fall 2010. Contents of dry matter (DM), total nitrogen (N_t) and NH_4^+-N as proportion of N_t are related to the slurry fresh substrate (FM). Total organic carbon (TOC) and NH_4^+-N are related to the slurry dry matter.

Substrate*	Farm	pH (H_2O)	DM % (FM)	N_t g kg^{-1} (FM)	NH_4^+-N % of N_t (FM)	NH_4^+-N g kg^{-1} (DM)	TOC g kg^{-1} (DM)
Spring application							
C	A	5.9	8.4	3.5	51.8	21.9	427.6
C lf	A	6.4	4.6	3.3	53.6	38.8	413.9
dC	A	7.3	5.7	3.4	50.4	30.3	399.9
dC lf	A	7.5	4.6	3.3	54.4	39.1	370.2
P	B	7.1	3.1	3.7	76.8	92.5	362.1
dP	B	7.8	5.7	5.1	65.4	58.3	350.0
dM	C	7.7	7.1	5.9	54.9	46.2	403.2
dM lf	C	7.6	5.3	5.6	66.1	69.9	379.9
Fall application							
C	A	6.0	7.9	3.1	42.7	16.8	459.7
C sf	A	8.4	18.5	4.4	6.2	1.5	455.0
dC	A	7.6	5.1	2.9	49.2	28.2	430.0
dC sf	A	8.5	17.3	3.8	22.7	5.0	473.3
P	D	7.0	1.1	1.4	72.2	90.5	381.2
dP	D	8.3	0.9	2.2	88.8	217.1	218.0
dM	C	8.4	5.9	5.8	63.6	62.1	511.1
dM sf	C	8.7	30.0	11.7	27.0	10.5	542.0

*C: cattle slurry, P: pig slurry, M: maize; d: digested; lf: liquid fraction; sf: solid fraction

Separation increased the pH value in the solid fraction by 1.2 units (p = 0.03, Holm-Sidak test) and the dry matter content by a factor 2.3 to 5.1 (p = 0.02, Holm-Sidak test). The digestates had slightly lower (not significant) DM content than the respective undigested slurries in three of the four analyzed samples (Table 2-1). Except for the separated solid fractions, all substrates had high NH_4^+-contents which accounted for 43 to 89% of the N_t content. Mechanical separation resulted in solid fractions with a lower NH_4^+-N to N_t ratio compared to the whole substrate (p = 0.03, Holm-Sidak test) (Table 2-1). The amount of NH_4^+ per kilogram of slurry (dry weight) varied widely between 1.5 and 217.1 g kg^{-1} without any differences regarding the substrate (cattle, pig or maize), digestion or separation. Separation strongly increased TOC concentration in the solid fractions (p = 0.03 Holm-Sidak test).

2.4.2 Temperature and wind speed

The mean daytime temperature during the measurement period in May was 24.5 °C, with daily maximum temperatures exceeding 30 °C on the first two days and with night temperatures above 12 °C. Maximum wind speed (2 m) reached 5.5 m s^{-1}, mean wind speed during daytime was 1.4 m s^{-1} (Figure 2-1a). In September, daily mean temperature during the measurement period was 16.1 °C with minimum values around 7°C at nighttime and with daily maxima of 21.5°C. Wind speed in September averaged 0.6 m s^{-1}, daytime peaks reached 2.9 m s^{-1} (Figure 2-1b).

No rainfall occurred in spring and only little in fall with 1.4 mm on the day of application and 1.1 mm the day after.

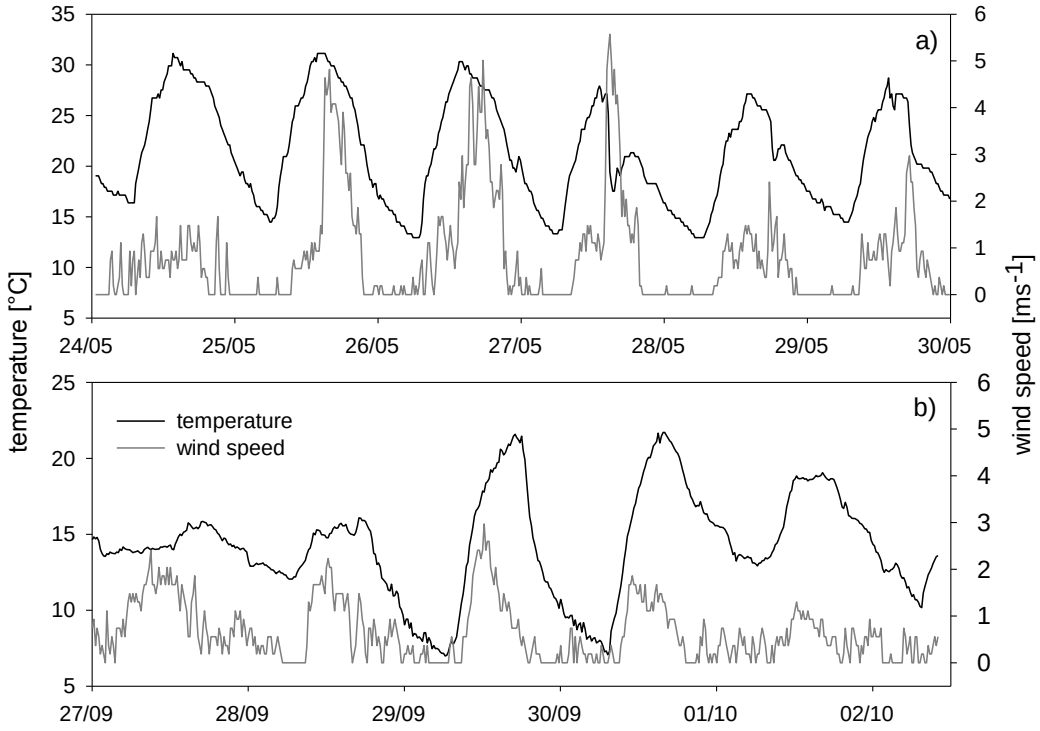

Figure 2-1: Air temperature and wind speed during the measurement periods in spring (a) and fall (b) 2010.

2.4.3 NH_3 emission

Spring application

Mean NH_3-N emission from the control treatment during the first and second day of measurements was 0.04 and 0.07 kg NH_3-N ha^{-1} h^{-1}. This low emission of the unfertilized control plot indicates a NH_3 drift with wind. However, compared to the NH_3 volatilization rates from the fertilized plots, this drift was small. All substrates showed similar emission patterns with highest volatilization rates during the first hours after application except for cattle slurry and the separated liquid fraction of cattle slurry. There, the initial peak of NH_3 volatilization was smaller and unlike the other treatments, highest emission rates were measured on the second day (Figure 2-2). Ammonia volatilization rapidly dropped at nighttime around 12 hours after application. Maximum NH_3-N emission rates during the first day were 0.4 to 0.9 kg ha^{-1} h^{-1}. Four hours after application, 3.0 to 16.3% of cumulative NH_3 losses (0.3 to 1.9% of NH_4^+-N applied) were volatized, after 8 hours between 4.6 and 29.5% (0.4 to 3.5% of NH_4^+-N applied) were lost.

Emissions increased again after the first night and were reduced but not completely stopped after incorporation with a rotary harrow 21 hours after application. In total, between 7.0 and 34.2% of the cumulative NH_3 losses (0.7 to 4.3% of NH_4^+-N applied) were emitted before incorporation.

During the following days, NH_3 volatilization showed a consistent pattern with increased fluxes at daytime and lower fluxes during the nights resulting in ongoing considerable losses on all treatments. After two days, more than half (51 to 61%) of cumulative losses had occurred. At the end of our monitoring time, 120 hours after application, NH_3 emissions from all treatments were close to zero.

Total NH_3-N losses ranged from 10.4 kg ha^{-1} (separated liquids of cattle slurry) to 18.6 kg ha^{-1} (maize digestate) (Table 2-2). Cumulative NH_3 emissions accounted for 5.8 to 13.3% of the total amount of NH_4^+-N applied. Digestates emitted significantly more NH_3-N in total and also in relation to the applied NH_4^+-N than the undigested substrates ($p < 0.01$), no effect of mechanical separation was found.

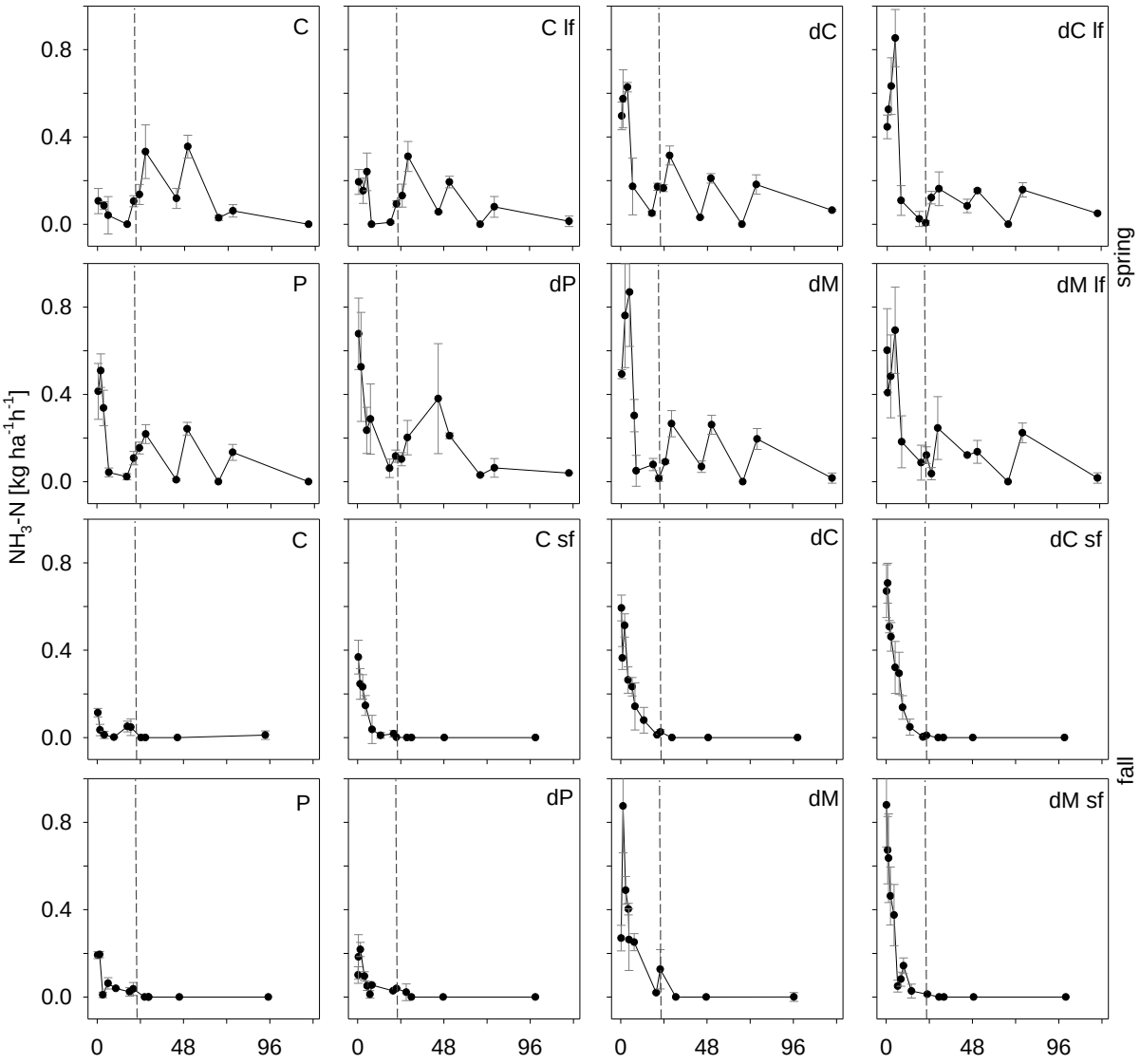

Figure 2-2: Ammonia volatilization rates (mean values and standard deviations) following the application of different slurry types and digestates to grain maize in spring (upper eight graphs) and fall (lower eight graphs) 2010. For the treatments with mechanical separation, the liquid fraction was applied in spring, the solid fraction in fall. Treatments: C: cattle slurry, P: pig slurry, M: maize, d: digested, lf: liquid fraction, sf: solid fraction. Slurry application at time = 0; slurry incorporation with rotary harrow in spring (15 cm) and a plow (30 cm) in fall after 21 hours, respectively is indicated by the dotted line.

Fall application

Low episodic NH_3-N emissions from the unfertilized control plot were 0.04 and 0.05 kg ha^{-1} h^{-1} on the first and second day, otherwise no emissions were measured (data not shown).

Ammonia emission patterns after slurry application in fall are shown in Figure 2-2. Again, highest emissions occurred directly after spreading and diminished rapidly within one day on all plots. Maximum emission rates of 0.9 kg NH_3-N ha^{-1} h^{-1} were similar to the spring season. Four hours after application, 17.1 to 62.7% of the cumulative NH_3-N loss (0.4 to 20.6% of NH_4^+-N applied) were

already emitted, after 8 hours these values increased to 17.1 to 81.8% (0.9 to 25.9% of NH_4^+-N applied).

Unlike incorporation with the rotary harrow in spring, NH_3 emission was nearly stopped by plowing after 21 hours. For six treatments, 90.5 to 99.8% of the cumulative NH_3-N losses had occurred until incorporation, but the treatments with maize digestate and raw cattle slurry still emitted 24.4 and 46.4 % of the respective cumulative NH_3-N loss the following days. For all treatments, emissions ranged between 0.8 and 5.8 kg NH_3-N ha^{-1} (Table 2-2).

Digestates emitted significantly more NH_3 than non-digested slurries ($p < 0.001$). Between 1.7 and 32.8% of the applied NH_4^+-N was lost by ammonia volatilization. Related to the applied NH_4^+-N, the separated solid fractions emitted more ($p < 0.001$) than the respective whole substrates.

Table 2-2: Cumulative NH_3-N losses following application of different slurry types in spring and fall calculated as total amount of NH_3-N ha^{-1} and as percentage of total $NH4^+$-N and total N (N_t) applied. The NH_3 monitoring period was 6 days in spring and 5 days in fall. Values in brackets indicate the standard deviation estimated by the Monte-Carlo simulation.

Substrate*	**Total N applied** kg ha^{-1}	**Total NH_3-N loss** kg ha^{-1}	**% of NH_4^+-N** applied	**% of N_t** applied
Spring application				
C	250.4	12.3 (1.7)	9.5 (1.3)	4.9 (0.7)
C lf	255.6	10.4 (1.5)	7.6 (1.1)	4.1 (0.6)
dC	257.1	17.2 (1.5)	13.3 (1.1)	6.7 (0.6)
dC lf	250.3	14.9 (1.3)	10.9 (1.0)	5.9 (0.5)
P	259.0	11.5 (1.1)	5.8 (0.5)	4.5 (0.4)
dP	295.9	17.0 (2.8)	8.8 (1.4)	5.8 (0.9)
dM	256.4	18.6 (1.8)	13.2 (1.3)	7.3 (0.7)
dM lf	243.9	17.9 (2.5)	11.1 (1.6)	7.4 (1.0)
Control	-	2.1 (0.6)	-	-
Fall application				
C	94.2	0.8 (0.5)	2.1 (1.2)	0.9 (0.5)
C sf	74.0	1.5 (0.3)	32.8 (7.5)	2.0 (0.5)
dC	93.8	3.4 (0.5)	7.3 (1.2)	3.6 (0.6)
dC sf	83.2	4.1 (0.4)	21.5 (2.3)	4.9 (0.5)
P	94.3	1.1 (0.2)	1.7 (0.3)	1.2 (0.2)
dP	97.3	1.6 (0.2)	1.8 (0.2)	1.6 (0.2)
dM	99.7	5.8 (0.8)	9.1 (1.2)	5.8 (0.8)
dM sf	85.8	3.6 (0.5)	15.3 (2.0)	4.1 (0.5)
Control	-	0.3 (0.2)	-	-

*C: cattle slurry, P: pig slurry, M: maize; d: digested; lf: liquid fraction; sf: solid fraction

2.5 Discussion

2.5.1 Substrate composition

Effect of primary substrate and digestion

Substrate composition is known to depend on animal diet, primary substrates (i.e. effluents from different animals, plant biomass), slurry storage, and slurry treatments such as digestion in biogas plants or slurry separation (Moller et al. 2002). Although cattle and maize substrates originated from the same farm in both seasons, respectively, the digestates originated from raw slurries of different seasons. Digestates collected in spring 2010 were derived from slurry of fall 2009, digestates collected in fall 2010 were derived from slurry of spring 2010. Consequently, there were farm-specific and seasonal variations in slurry properties.

All slurries had a high NH_4^+-N content and can therefore be considered valuable N fertilizers. The results agree with the general finding that NH_4^+-N accounts for 50 to 70% of total slurry N (Christensen et al. 2009; Moller et al. 2000; Sommer & Husted 1995). Most of the digested cattle and pig slurry samples of our study showed typical changes in their chemical composition after digestion, i.e. a higher pH value (Pain et al. 1990b) and a reduced dry matter and total organic carbon content of digested cattle slurry (Pain et al. 1989; Rubaek et al. 1996). Digestates applied in fall showed increased availability of NH_4^+-N (Rubaek et al. 1996). However, in some samples, e.g. pig slurry and digested pig slurry from the spring season, this general effect of digestion was not observed. This may be attributed to the fact that digested pig slurry was sampled in different seasons, i.e. during different stages of pig fattening and multiple phase feeding therefore probably having a lower initial N content in fresh slurry.

Effect of separation

For all substrate types, mechanical separation resulted in solid fractions with strongly increased dry matter and organic matter content. Our results confirm the expected N partitioning after mechanical separation with higher N_t and lower NH_4^+-N content of the separated solid fractions. After separation, the liquid fraction can still contain large amounts of N, around 45 to 80% of N in the liquid phase is present as NH_4^+-N (Möller & Müller 2012). Separation of the substrates produced a liquid fertilizer with easily available NH_4^+-N (> 53% of N_t) and a solid fertilizer with a high potential for soil organic matter replenishment and addition of organically bound nutrients (e.g. nitrogen, phosphorus). In general, solid separation residues contain a relatively high amount of organic N (Moller et al. 2000) but as their composition varies greatly, the solid fraction can also contain considerable amounts of mineral nitrogen (Möller & Müller 2012).

2.5.2 NH_3 emission

Total NH_3 losses

The cumulative NH_3-N losses related to the applied NH_4^+-N of 5.8 to 13.3% in spring were relatively low compared to other studies, where proportions of up to 35% of the applied NH_4^+-N were volatilized after application with trail hoses to growing crops (Balsari et al. 2008; Pacholski et al. 2010; Quakernack et al. 2012). The EMEP guidebook 2009 for agricultural emission inventories (EEA 2009) reports an average NH_3 emission factor by volatilization of 55 and 40% of the applied total ammoniacal nitrogen (TAN = NH_3-N + NH_4^+-N) content for broadcast application (without incorporation) of cattle and pig slurry, respectively. Band application in growing crops is assumed to reduce NH_3 losses by about 30% (depending on crop height) and immediate incorporation can reduce emissions by up to 90%. However, the recent review of Sintermann et al. (2012) showed that the reported emission factors for splash plate application and band spreading vary considerably.

The relatively low emissions in our study were probably not only a result of mitigation measures such as band application and incorporation but also of crust formation during the day of application. Crusts form from slurry dry matter which remains on the soil surface (Sommer et al. 2003) and can lower rates of NH_3 volatilization considerably (Misselbrook et al. 2005; Thompson et al. 1990). Apparently, the fast crust formation effectively reduced the NH_4^+ pool prone to volatilization by forming a natural sealing. We did not observe any major differences in infiltration into the soil between the different substrates in spring. Infiltration into the fine textured soil in this study was impeded by high soil moisture content, in particular in spring, and no cracks were visible. During spring application, slurry remained as liquid bands on the soil surface for several hours before infiltration.

Even though the observed NH_3 losses were lower than the average EMEP default values, they still affected the value of the slurries as N fertilizer because up to one third of the easily plant available NH_4^+-N was lost after fall application.

Temporal volatilization patterns

The majority of our treatments exhibited the typical NH_3 emission pattern for slurries and digestates rich in NH_4^+-N with highest emissions directly after slurry spreading (Gordon et al. 2001; Meade et al. 2011; Möller & Stinner 2009; Wulf et al. 2001). However, during the spring season, substantial NH_3-N loss still occurred even after harrowing. The more persistent NH_3 emission in spring revealed distinct day-night changes of NH_3 emission. This effect was associated with high daily temperatures, solar radiation, and relatively high wind speeds, which are crucial factors for NH_3 volatilization (Braschkat et al. 1997; Sommer & Hutchings 2001; Sommer et al. 1991).

Volatilization of NH_3 from animal slurries may however not only be driven by the content of TAN in the liquid phase of the slurry and pH value, but also by urea. The TAN in animal manure originates from the hydrolysis of urea which is present in urine (Sommer & Hutchings 2001). However, as urea is rapidly hydrolyzed and slurry is usually stored for several weeks prior to application, this process of hydrolysis is nearly complete before the slurry is transported to the field (Sommer et al. 2003). Nevertheless, we cannot exclude that some urea still present in the animal slurry after application to the field can be hydrolyzed by the soil enzyme urease during high temperatures and may be responsible for some of the emissions still occurring at later stages of the experiment.

Influence of slurry incorporation on NH_3 emissions

Soil mixing with a rotary harrow in spring did not stop NH_3 emissions whereas emissions completely disappeared after plowing in fall. Direct incorporation of slurry is suggested to minimize NH_3 losses after application (Ni et al. 2012; Sommer & Hutchings 2001; Wulf et al. 2002a). However, the rotary harrow produced a loose soil structure and enlarged the soil surface which was affected by the substrate which, in turn, was not completely removed from the soil surface. The effect of harrowing on NH_3 emission largely depends on proper mixing of soil and manure, which affects the amount of slurry exposed to the atmosphere and the extent of chemical interactions of slurry and soil (i.e. changes in pH and NH_4^+-concentration). Webb et al. (2010) recommended incorporation of manure and slurry with the plow as most effective tool to reduce NH_3 losses. Other techniques of incorporation (e.g. by disc or harrow) were less effective in NH_3 emission abatement.

Influence of slurry digestion on NH_3 emissions

Digestion increases the NH_4^+-N content and the pH value of the substrate, which may increase the risk of NH_3 loss. On the other hand, digestion reduces viscosity, which favors slurry infiltration and may decrease NH_3 volatilization (Sommer & Husted 1995; Wulf et al. 2002a). Our results support the dominance of the NH_4^+-N effect because digested animal slurries emitted 40 to 50% (spring) and 50 to 330% (fall) more NH_3 after slurry application than undigested slurries. In our study, initial NH_3 emission rates after digested slurry application were 20 to 200% higher than from undigested slurries and up to 300% higher from digested maize than from digested animal slurries. After the first night, NH_3 emission rates among the treatments adapted. In consequence, only immediate and complete incorporation can effectively mitigate NH_3 emissions from digested slurry and even more from digested maize substrate and incorporation of the substrate more than 12 hours after spreading has only a slight effect on N losses (Möller & Stinner 2009). Our results agree with earlier findings (Ni et al. 2012; Pacholski et al. 2010) of enhanced NH_3 emission from mono-fermented biogas digestate of energy crops or co-fermented digestate of energy crops and animal slurry. Although data availability

from field studies with mainly energy plant-derived digestates is scarce, the results indicate a remarkably high NH_3 emission potential of those substrates.

Influence of separation on NH_3 emissions

The evaluation of the total effect of separation has to integrate emissions from both the liquid and the solid fractions of the respective substrate. We found no evidence that mechanical separation reduced NH_3 emissions from slurry application, but measured even higher emissions following the application of the solid fraction in fall.

Due to the high NH_4^+-N content of the separated solid fraction its potential to emit NH_3 is probably much higher than the NH_3 emission potential of other solid manures, e.g. farmyard manure. (Hansen et al. 2006; Möller & Müller 2012). In our study, the separated solid fractions showed 1.7 to 15.6-fold higher NH_3 losses related to applied NH_4^+-N than the respective non-separated substrates. The results are in line with Dinuccio et al. (2012) who found that the solid fraction was the main source of NH_3 losses during both storage and after land application of separated pig slurry. We attribute this result to the high pH and structure of the solid fraction, which favored NH_3 volatilization. Due to the high dry matter content and the accumulation of structural organic matter, the solid fractions remained at the soil surface and exposed a large surface area to solar radiation and wind turbulence. Since they still contained considerable amounts of NH_4^+-N, relative emissions of NH_3 were large.

Although there is a lack of data from field investigations with separated solid fractions, the results demonstrate that band spreading of the solid slurry fraction does not effectively mitigate NH_3 emissions. Fast incorporation of slurry or digestates should be employed to both fractions (solid and liquid) of separated slurry to avoid NH_3 emissions.

2.6 Conclusions

Our study provides systematic field data of NH_3 volatilization from the three most common types of slurries, digestates and separated fractions in warm temperate climate applied according to common agricultural practice. The fraction of NH_4^+-N in slurries and digestates lost as NH_3 was relatively low in both spring and autumn applications (1.7 to 13.3% for non separated slurries). Apparently, NH_3 emissions were limited by the combination of band application and crust formation together with soil incorporation. Digestates emitted more NH_3 than fresh slurries, in particular during the first eight hours. In addition, the maize-based digestate exhibited the highest NH_3 emission rates directly after application. Specific emission factors need to be developed for pollution inventories to adequately capture NH_3 emissions from digestates.

Separated solid fractions had noticeable higher NH_3-N losses in relation to NH_4^+-N content than non-separated slurries. The solid fraction does not infiltrate into the soil surface so that only mechanical incorporation can reduce NH_3 emissions. This indicates that all types of slurry, digestate or solid fractions with considerable amounts of reactive TAN must be quickly incorporated into soil. Our results suggest that the emission potential in relation to NH_4^+-N content for separated solid fractions is higher than for non-separated slurries. In contrast, the NH_4^+-N related NH_3 emissions from fresh slurries and separated liquid fractions were comparable.

Only complete slurry incorporation by plow effectively stopped NH_3 emissions while shallow harrowing was not effective. The study underlines the importance of fast and complete incorporation of the slurries and digestates and their solid and liquid fractions. This calls for legal steps to make immediate incorporation mandatory for all types of organic manures. Reduced NH_3 emissions help to retain the fertilizer value of the digestate or slurry and hence allow an improved fertilization management for the farmers.

3 Contribution of N_2O and NH_3 to total greenhouse gas emission from fertilization – results from a sandy soil fertilized with nitrate and biogas digestate with and without nitrification inhibitor

3.1 Abstract

Fertilization with biogas residues from the digestion of energy crops is of growing importance. Digestate from silage maize *(Zea mays L.)* is a new fertilizer with a high potential for ammonia (NH_3) and nitrous oxide (N_2O) emission. The aim of this study was to determine the effect of different maize fertilization systems (180 kg N ha^{-1} in form of calcium nitrate (MIN), biogas digestate from maize (DIG) and biogas digestate from maize mixed with the nitrification inhibitor *Piadin* (DIG+NI)) on the emission of NH_3 and N_2O from a sandy soil and to assess the total greenhouse gas emission of these fertilization systems. The study is based on a randomized field plot experiment in central Germany and an experimental period of a full year. Annual N_2O-N emission was generally low (0.21 (MIN) to 0.37 (DIG) kg N ha^{-1}) without differences between treatments. The application of *Piadin* reduced N_2O emissions by 37 and 62% during the weeks following digestate application but there was no effect on the annual N_2O emission. NH_3 emission was only significant for treatments fertilized with digestate. It was not affected by *Piadin* and accounted for 27% (+ NI) and 29% of the applied ammonium. Total greenhouse gas emission was dominated by indirect N_2O losses for the treatments fertilized with maize digestate, and emissions from fertilizer production were the most important greenhouse gas source of the calcium nitrate treatment. Our results show the high potential of digestate from maize as a new source of NH_3 emission. Mitigation measures are essential to save the value of this new fertilizer type and to reduce atmospheric and environmental pollution by direct emission of NH_3 and indirect emission of greenhouse gases.

3.2 Introduction

Biogas residues have been growing in importance as a valuable fertilizer in agriculture since the number of biogas plants is increasing in many European countries. The greatest expansion of biogas production has occurred in Germany where the passage of the first Renewable Energy Sources Act in 2000 induced an increase in the number of biogas plants from 1,000 plants in the year 2000 to more than 7,500 plants in 2012 (FNR 2013). Meanwhile nearly one third of the total maize growing area in Germany is used for biogas production and maize silage is the most important substrate of biogas production (Scheftelowitz 2013). Digestion in biogas plants changes the chemical composition and results in digestates with lower organic carbon (C) content, increased ammonium (NH_4^+) content, and increased pH value (Rubaek et al. 1996). These changes influence the value of digestates as fertilizer and they alter the risk of ammonia (NH_3) and nitrous oxide (N_2O) emission following fertilization with digestates. Despite the increasing importance of digestates from energy crops as a new N fertilizer in agriculture, there are only a few studies on their effects on the emission of NH_3 or N_2O.

Results from field studies on surface application of digested cattle or pig slurries (Amon et al. 2006; Möller & Stinner 2009; Nyord et al. 2012) reflect the high potential of digestate for NH_3 emission, which can be explained by the high pH and NH_4^+-N content. Up to 40% of the NH_4^+-N applied with the digestates was lost as NH_3 (Nyord et al. 2012). Also mono- or co-fermented plant material was shown to induce considerable NH_3 emissions (Gericke et al. 2011; Quakernack et al. 2012). These results stress the importance of mitigating NH_3 emission from this growing NH_3 source by using adequate application techniques such as immediate incorporation (Vandermolen et al. 1990) or application with trail hoses in growing crops (Sommer & Hutchings 2001).

Liquid manure application favors the emission of N_2O from soils with its high content of NH_4^+-N in combination with bioavailable organic matter which promotes N_2O production by nitrification and denitrification (Dendooven et al. 1998; Flessa & Beese 2000; Kaiser & Ruser 2000). Due to the decomposition of organic material during digestion, digestates contain less metabolizable organic C which may reduce the potential for denitrification after application (Petersen 1992). Some results on N_2O emission in systems fertilized with digested animal slurries suggest that anaerobic digestion can reduce the risk of N_2O emissions (Chantigny et al. 2007; Petersen 1999; Vallejo et al. 2006; Wulf et al. 2002b) or denitrification losses (Rubaek et al. 1996) from applied substrates. However, results are not consistent as Amon et al. (2006) reported no effect of digestion on N_2O emissions and Thomsen et al. (2010) even found a trend for increased N_2O emission from digestates. Möller and Stinner (2009) reported remarkably increased N_2O emissions after shallow injection of digestates and attributed this to enhanced denitrification induced by the higher supply of readily available mineral nitrogen. However, measurement periods of N_2O emissions only covered a few weeks and thus displayed only short-term effects of fertilization.

The use of nitrification inhibitors (NIs) has been proposed to reduce N_2O emissions from liquid manure and synthetic N fertilizers which contain or form NH_4^+-N (Prasad & Power 1995; VanderZaag et al. 2011) and thereby increase the N use efficiency of fertilizers (Subbarao et al. 2006). Most NIs retard microbial oxidation of NH_4^+ by depressing the activities of nitrifiers in soil. Thus, mineral N is stabilized in the rather immobile form of NH_4^+ instead of being transformed to nitrate (NO_3^-), a highly mobile form which provides a much greater potential for leaching from the rooting zone or gaseous emission in the form of N_2O (Subbarao et al. 2006). Akiyama et al. (2010) reported a mean reduction of N_2O emission by 38% as a result of the application of NIs. However, their evaluation is mainly based on short-term effects of NIs and there are only a few studies covering a whole year (Dobbie & Smith 2003a; McTaggart et al. 1997; Parkin & Hatfield 2010; Pfab et al. 2012) which allow a more comprehensive evaluation of emissions from fertilization systems with and without NIs. Nitrification inhibitors were also shown to reduce N_2O emissions after the application of different animal slurries to grassland and arable soils (Meijide et al. 2007; Merino et al. 2005), but again long-term

observations covering a whole year are missing. We found no study on the effect of NI on N_2O emission from digestate application.

For the integrated evaluation of the total greenhouse gas (GHG) emission from fertilization, both N_2O and NH_3 have to be taken into account. Complex interactions exist between emission of NH_3 and N_2O and some measures to mitigate the emission of one gas may enhance the emission of the other compound (Ferm et al. 1999; Webb et al. 2010). Although the evaluation of mitigation options requires a system approach including both gases and all relevant emissions, we found no study where N_2O and NH_3 emissions from maize derived digestates were determined in the same experiment allowing the direct evaluation of their contribution to the total greenhouse effect of fertilization.

We conducted a field experiment on the influence of different fertilization systems (i.e., fertilization with calcium nitrate, digestate from maize, digestate from maize with NI) on the emission of N_2O and NH_3 with the aim of improving the knowledge on the environmental impact of this new fertilizer from biogas production, and of evaluating the integrated effect of a NI on these emissions.

The objectives of the study were (1) to quantify total emission of N_2O and NH_3 from maize field plots with different types of N fertilization ($Ca(NO_3)_2$ and biogas digestate, 180 kg N ha^{-1}); (2) to determine the influence of a nitrification inhibitor (*Piadin*) on the emission of N_2O and NH_3 for the treatments fertilized with biogas digestate, and (3) to evaluate the importance of N_2O and NH_3 emissions for the total greenhouse gas balance of the different fertilization strategies. Our study is based on a randomized field plot experiment and a measurement period of a full year to capture both short term effects of fertilization treatments and their influence on the annual emission.

3.3 Materials and methods

3.3.1 Study site

The experimental site was located on the research area of the Julius Kühn Institute, Braunschweig, central Germany (52°18’ N, 10°26’ E, 77 m a.s.l.). The mean annual temperature is 9.1°C with an annual precipitation of 617 mm (German climate service). The soil type is a cambisol, the texture of the topsoil (30cm) is a silty sand (8% clay, 30% silt, 62% sand) with a pH (H_2O) value of 6.6 and an organic carbon content of 9 g kg^{-1}.

Experimental set-up and field management

A fully randomized field plot experiment (block design, plot size of 12 x 12 m) with maize cropping and four replicates of each fertilization treatment was established in April 2011. The following fertilization treatments were established:

1. Fertilization with $Ca(NO_3)_2$, fertilization rate 180 kg N ha^{-1}, one application date (MIN)
2. Fertilization with biogas digestate from silage maize and addition of a nitrification inhibitor (*Piadin*), fertilization rate 180 kg N ha^{-1}, split application (DIG+NI)
3. Fertilization with biogas digestate from silage maize, fertilization rate 180 kg N ha^{-1}, split application (DIG)

Maize (Zea mays L., "Atletico") was sown on April 28, 2011 on all plots. Mineral N fertilizer ($Ca(NO_3)_2$) was applied in the MIN treatment at a rate of 180 kg N ha^{-1} on May 17th. Application of biogas digestates (DIG and DIG+NI) was split. The first application (82 kg total N ha^{-1}) was carried out with trail hoses on bare soil on April 26, 2011, two days before the sowing of the maize. Digestate was incorporated with a rotary harrow the day after the first application to a depth of approximately 15 cm. Another 97 kg N ha^{-1} were applied with trail hoses between crop rows on June 6, 2011 (39 days after seeding).

Biogas digestate was collected from a biogas plant about 20 km south of Braunschweig which was fed with maize silage. Characteristics of the biogas digestate are given in Table 3-1. Digestate samples were analyzed by an external contract laboratory for agricultural analytics (LUFA North-West, Oldenburg, Germany).

Table 3-1: Composition of the fresh digestate and total N application rate for the fertilization in April (before seeding) and in June (application between maize rows).

Date	application rate	pH	dry matter	N_{tot}	NH_4-N	C
	kg N ha^{-1}		g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}
04/26/2011	82	7.6	67.4	3.7	1.8	28.0
06/06/2011	97	7.7	73.4	4.6	2.2	30.2

For the DIG+NI treatment, the nitrification inhibitor *Piadin* (SKW, Piesteritz, Germany) was mixed with biogas slurry directly before application on both application dates (application rate: 6 l ha^{-1}). *Piadin* consists of the inhibitory agents 3-Methylpyrazol (1.5%) and Triazol (3%) which are diluted in ammonium nitrate urea solution.

Because of the rather dry conditions in early summer 2011, all field plots were irrigated with 30 mm of water on June 21st. Maize grain was harvested on November 1, 2011. All crop residues remained on the field and were mulched prior to plowing on November 2nd. In the end of March 2012 plowing was repeated and spring wheat was sown.

3.3.2 Gas flux measurements

Fluxes of N_2O were measured using the closed chamber method (Hutchinson & Mosier 1981). We used white, not translucent rectangular chambers with an edge length of 0.65 x 0.65 m and a height of 0.15 m. The chambers were placed on pre-installed frames which were pressed 0.06 m into the soil. These base frames were removed only for digestate spreading, soil preparation and harvest and they were then re-installed immediately at the same location. Chambers were placed between crop rows and covered nearly the whole area between plants (distance between maize rows was 0.75 m). At each sampling date, chambers were closed and sealed air tight with rubber bands for one hour. Four gas samples were taken with evacuated glass vials (100 ml) with a Teflon valve during the enclosure period (sampling interval of 20 minutes).

Concentration of N_2O in the gas samples was measured with a gas chromatograph (GC 2014, Shimadzu, Duisburg, Germany) with a back-flush system equipped with a ^{63}Ni electron capture detector and a pressure controlled auto-sampler unit. A detailed description of the set-up and function of the system is given in Loftfield et al. (1997). The gas chromatograph was operated at a column temperature of 70°C and nitrogen (27 ml min^{-1}) was used as carrier gas. The precision of N_2O analysis was checked weekly by repeated determination of a standard gas with ambient N_2O concentration (320 ppb) and was consistently around 6.4 ppb (2%).

Field measurements were conducted from April 2011 to April 2012 in weekly intervals. After fertilizer application, harvest and tillage, daily gas emission measurements were performed for one week. This sampling design followed the recommendations of Flessa et al. (2002) for event-driven higher sampling frequency and a constant weekly sampling throughout the year. Flux calculation followed the linear regression of the gas concentration of the four samples over time (Ruser et al. 1998). Flux rates were expressed as means (n = 4) with standard deviation from the replicated field plots. Cumulative N_2O emissions were calculated from the flux values of each plot with linear interpolation between two measurement dates. Nitrous oxide emissions were converted into CO_2-equivalents (CO_2-eq) using the global warming potential for N_2O (289) on a basis of a time horizon of 100 years (IPCC 2006).

3.3.3 Soil and plant analysis

Soil mineral nitrogen ($N_{min} = NO_3^- + NH_4^+$) in 0 to 20 cm depth was measured at each date of N_2O flux determination unless the soil was completely frozen. Fifty grams of fresh soil were extracted with 200 ml of a 0.01 M $CaCl_2$ solution by shaking for one hour. Mineral N concentrations of the extracts were determined by using a photometric continuous flow analyzer (SA 5000, Skalar Analytical B.V., Netherlands).

Soil moisture content was analyzed gravimetrically after drying the soil at 105°C until constant weight. Water-filled pore space (WFPS) was calculated from the gravimetric water content and the soil bulk density determined on the field plots with undisturbed soil samples taken with stainless steel cylinders (100 cm^3).

Dry matter, C and N content of maize plants as a whole and grains were determined after drying at 105°C for determination of dry matter, and at 60°C for analysis of C and N. Samples were milled and analyzed with a CN auto-analyzer (LECO Instruments, St. Joseph, USA).

3.3.4 Ammonia volatilization measurement

Ammonia losses after application of the digestates were determined by combining two different measurement systems (passive samplers and Draeger-Tube-Method) as described and evaluated by Gericke et al. (2011). The combination of the two methods allows the determination of cumulative NH_3 fluxes on the basis of NH_3 trapping in passive samplers.

The Draeger-Tube-Method (DTM) is a variant of an open dynamic chamber system which was successfully used in several experimental studies on field plots (Pacholski et al. 2006; Quakernack et al. 2012; Roelcke et al. 2002). The measurement system consists of four tinplate cups (surface area 416 cm^2, height 15 cm) with copper tubes as air inlet and outlet in the lid. Via Teflon tubes, the four cups are connected and form one measurement unit. For DTM measurements, stainless steel soil rings were installed into the upper soil to ensure tight sealing between measurement chambers and the soil surface. During the NH_3 measurement, air is sucked by a pump which is connected to the air outlet pipe. Thus it conducts the air from the cups through the ammonia-sensitive Draeger indicator tube (Draeger Safety AG, Lübeck, Germany) where the concentration of ammonia gas in the air is immediately displayed. From the displayed NH_3 concentration, a NH_3 flux rate was calculated and adapted to ambient air velocity with the calibration approach of Pacholski et al. (2006). Since the measurements were conducted after digestate application on bare soil in April and in growing crop in May, we used the calculation recommended for winter conditions and growing crop, including ambient air velocity in 2 meters height. Single measurements with the DTM can be performed within about 5 minutes. We used the mean of three consecutive flux determinations with the DTM to describe NH_3 emission at each sampling time. Directly after digestate application, NH_3 concentrations were measured every two hours and measurement frequency was decreased over time. We determined quantitative NH_3 emissions with the DTM on one replicate of each treatment.

Qualitative differences between the treatments were determined by using passive samplers which continuously trapped NH_3 from the air and provided integrative values for NH_3 losses. One passive sampler was installed at a height of 0.15 m in the centre of each 12 x 12 m plot. The samplers consisted of a modified polyethylene square bottle with circular holes in the side (for detailed

description refer to Gericke et al. (2011)), filled with 20 ml of 0.05 M H_2SO_4. The sampler solution was changed in intervals of 3 to 24 hours. The collected samples were stored frozen until the trapped NH_4^+ was analyzed with a continuous flow analyzer (SA 5000, Skalar Analytical B.V., Netherlands).

To calculate absolute NH_3 losses (kg NH_3-N ha^{-1}) from the amount of NH_3 trapped by the passive samplers, a transfer coefficient was used as proposed by Vandre and Kaupenjohann (1998). The transfer coefficients were calculated as the ratio of cumulated NH_3 collected by passive samplers and cumulated absolute NH_3 losses (kg N ha^{-1}) determined by the DTM at the end of each experimental campaign (Gericke et al. 2011). For each treatment, one conversion factor was calculated and used for all plots of the respective treatment. Cumulative NH_3-N losses (kg N ha^{-1}) were calculated by linear interpolation between two subsequent measurements.

Ammonia measurement lasted 2 days following the first digestate application and 4 days during the second campaign and was started immediately after application. Volatilization of NH_3 was measured in all treatments (MIN, DIG, DIG+NI) following digestate application at DIG and DIG+NI. Ammonia emission from the MIN treatment was not determined directly after application of the $Ca(NO_3)_2$ since it is known that nitrate addition does not induce significant NH_3 emission under the given soil conditions. Ammonia loss from the MIN treatment measured after digestate application provides information on the importance of possible NH_3 drift from the digestate treated plots.

We calculated indirect N_2O emission from deposition of emitted NH_3-N using the IPCC (2006) guidelines (EF_{N2O} = 0.01 kg N_2O-N kg^{-1} NH_3-N).

3.3.5 Calculation of greenhouse gas (GHG) balance

The two fertilization systems (mineral fertilization and fertilization with digestates) differ regarding i) direct N_2O emissions (i.e. measured N_2O emissions), ii) indirect N_2O emissions (i.e. induced by observed NH_3 emissions), iii) emissions from diesel consumption by the tractor for the application of the respective fertilizer and iv) emissions from production of synthetic N fertilizer. All these sources of direct and indirect emissions have to be taken into account in order to consider the treatment effects on greenhouse gas emissions as a whole (Thomsen et al. 2010; Wulf et al. 2002b) All emissions were converted into CO_2-equivalents (CO_2-eq.) according to the (IPCC 2006) guidelines to obtain a comparable unit.

Emissions from N fertilizer production were relevant in particular for the MIN treatment. As NH_3 losses can remarkably reduce the value of biogas residues as N fertilizer, we also considered the emissions related to this lost fertilizer value in terms of mineral N fertilizer equivalents. This approach assumes that mineral N which is lost as NH_3-N has to be substituted by the input of mineral N fertilizer (Thomsen et al. 2010). The mean emission of CO_2-eq from production of $Ca(NO_3)_2$ was assumed to be 9.6 kg CO_2-eq kg^{-1} N, (Brentrup & Pallière 2008). Data for diesel consumption and

related emissions of CO_2-eq were derived from the KTBL diesel-consumption online calculation tool (http://daten.ktbl.de/dieselbedarf/main.html). The calculation includes diesel consumption from broadcast application of calcium nitrate (MIN) and from digestate application with trail hoses and subsequent incorporation with a rotary harrow (DIG treatments). Diesel consumption from harvest and plowing was not included in the comparison of the fertilization strategies as it was similar for all treatments. Estimation of GHG emission from diesel included emissions from the supply chain and from diesel burning in the tractor's combustion engine. The mean emission of CO_2-eq per unit diesel consumed was assumed to be 3.01 kg (GaBi Professional Database Version 4.131).

3.3.6 Statistical evaluation

Regression analysis of N_2O fluxes

We used regression analysis to assess significant differences of treatments in different time periods. The time periods were defined as follows: The measurement year was divided into three periods to compare the effect of the fertilization treatments on N_2O emission direct after fertilizer applications and during the rest of the year. Two periods covered the weeks following the respective digestate applications (*Period 1*: 04/26/11 – 06/05/11, *Period 2*: 06/06/11 – 08/05/11) and one period covered the rest of the year including winter time (*Period 3*: 08/06/11 – 04/26/12).

Since the flux measurements are time series data with repeated measures at the same positions, they violate the assumption of independence needed for inference from ordinary least squares regression. Thus, we used linear mixed effects models as implemented in the package *nlme (Pinheiro et al. 2012)* for the R language (2.15.0, The R Foundation for Statistical Computing) following the methods detailed in Zuur et al. (2009). This allows modeling repeated measures by using a random intercept per plot as well as autocorrelation of the residuals by using a first order autoregressive (AR1) autocorrelation structure. Since residuals deviated strongly from normal distribution for the N_2O data, as assessed using residual plots, N_2O fluxes were log-transformed. This is common practice and appropriate for N_2O data (e.g. Folorunso & Rolston 1984).

In dry periods with low emission activity there were a few sampling dates where single chambers indicated a net uptake of atmospheric N_2O (negative flux rates). These small negative fluxes were highly uncertain and we found no sampling date where all plots and treatments were a significant sink for atmospheric N_2O. To account for the few negative fluxes we transformed the data using log10 (N_2O-flux + 9 µg m^{-2} h^{-1}). However, this was still not sufficient to achieve homogeneity of residual variance in the mixed effects model. Therefore, we modeled variance heterogeneity using standard errors of N_2O fluxes (obtained from flux calculation) as a variance covariate (i.e., fluxes measured less precisely can deviate more strongly from the model then fluxes measured more

precisely). The fixed effects part of the model consisted of the factors *treatment* and *period* and their interaction. Finally, pair-wise comparisons were conducted using Tukey's test as implemented in package *multcomp* (Hothorn et al. 2008). The level of significance was set to $p < 0.05$.

Ammonium and nitrate concentration data were analyzed with the same model framework as N_2O fluxes. The concentrations had to be log10-transformed to achieve normality of residuals and variance homogeneity.

Comparison of annual N_2O and cumulated NH_3 emissions

With the four cumulated N_2O emissions of the replicated plots, analysis of variance (ANOVA) was run to identify differences in the annual emissions between the treatments. If ANOVA indicated differences, a Tukey test was calculated for pair-wise comparisons. For all tests, the level of significance was set to $p < 0.05$.

Total NH_3 volatilization for each measurement period were calculated and compared in the same manner. To analyze the effect of application date and treatment on NH_3 emissions from the organic fertilizer treatments, a two-factorial-ANOVA and subsequent Tukey tests were calculated.

3.4 Results

3.4.1 N_2O emissions

Fluxes of N_2O-N were generally below 100 µg N_2O-N m^{-2} h^{-1} (Figure 2-1) with a mean flux of 4.3 and a median flux of 2.2 µg N_2O-N m^{-2} h^{-1} for the three treatments. The treatments with digestate application (DIG, DIG+NI) showed a distinct N_2O emission peak following fertilization that declined within two weeks. Application of calcium nitrate did not induce a direct increase in N_2O emission. All treatments showed increased emission rates following irrigation on June 21st (Figure 3-1). Directly after irrigation fluxes reached mean values of 94.5 µg N_2O-N m^{-2} h^{-1} on the DIG treatment, 23.5 µg N_2O-N m^{-2} h^{-1} on the DIG+NI treatment and 12.6 µg N_2O-N m^{-2} h^{-1} on the MIN treatment plots. Soil rewetting by irrigation resulted in a short-term increase of N_2O emissions. From mid-July 2011 to April 2012 all treatments exhibited N_2O fluxes below 10 µg N_2O-N m^{-2} h^{-1}. We have not observed increased N_2O fluxes following heavy rainfall events (> 15 mm day^{-1}), during winter or frost-thaw events.

Regression analysis of the influence of the treatment showed significant differences in the mean N_2O fluxes depending on the period. For all treatments mean emission was higher in *Periods 1* and *2* (periods following digestate application) than in *Period 3* without fertilizer application ($p < 0.05$). Additionally, the DIG treatment emitted more N_2O in *Periods 1* and *2* than the MIN treatment ($p < 0.001$). There were no significant differences between the MIN treatment and the DIG+NI treatment

in all periods. The addition of *Piadin* led to reduced mean N_2O fluxes following digestate application ($p < 0.05$) compared to the DIG treatment.

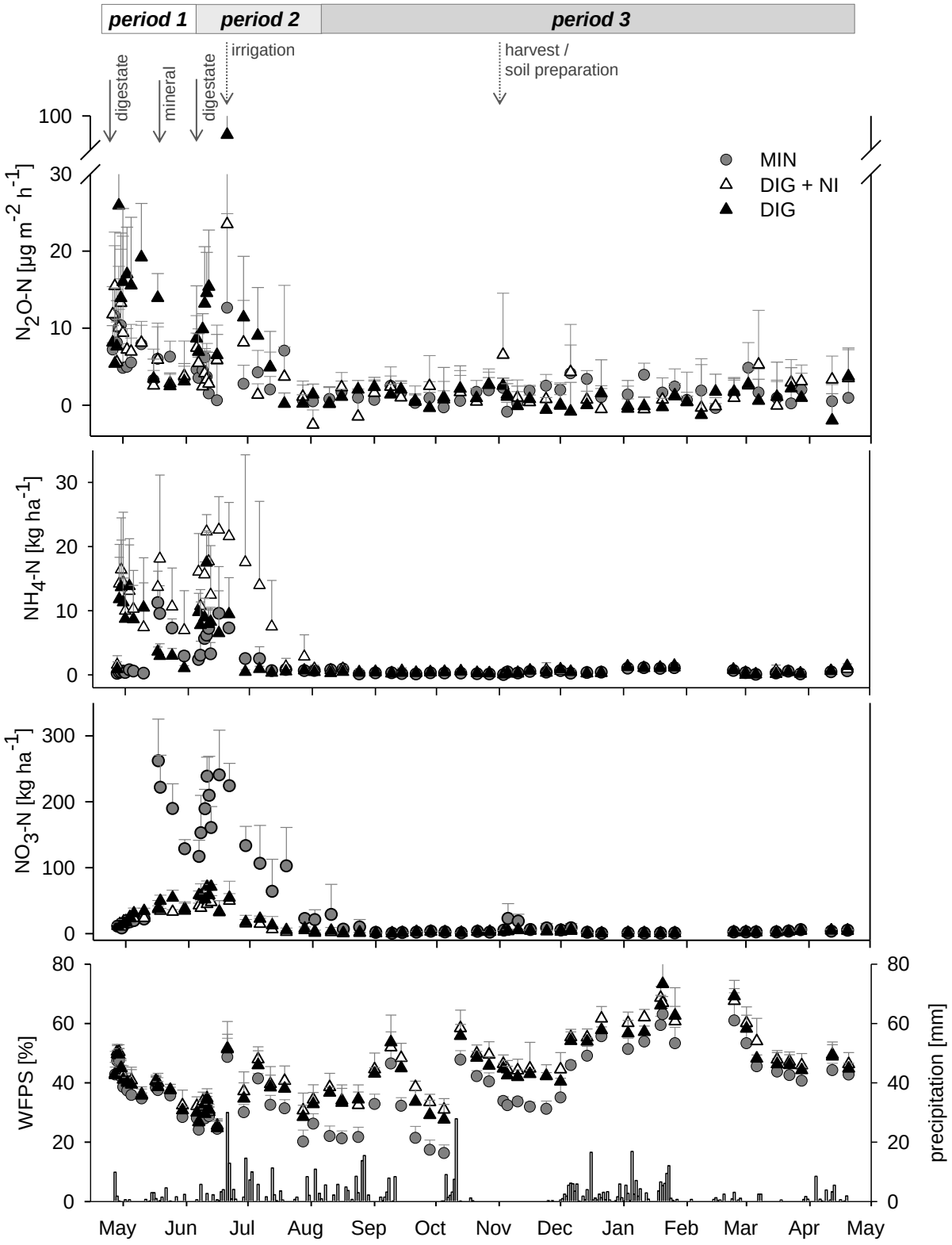

Figure 3-1: Time course of N_2O emission, soil NH_4^+-N and NO_3^--N concentration (0-20 cm), soil water-filled spore space (WFPS, 0-20 cm) and precipitation for the investigation period (April 2011 to April 2012). Data points represent means with standard deviation (only the upper parts of the error bars are illustrated, n = 4).

Cumulative N_2O emissions for each period and treatment are shown in Table 3-2. The two periods following digestate application *(Periods 1* and *2,* together 14 weeks) contributed 56% (MIN), 86% (DIG+NI) and 64% (DIG) to the annual N_2O loss.

Even if there were some differences in N_2O emission between fertilization treatments following fertilizer application, these differences leveled out at the annual time scale (Table 3-2). Yield related N_2O emission calculated from maize grain yield and the annual N_2O emission was higher for the treatments fertilized with digestate than calcium nitrate (Table 3-2).

Table 3-2: Fertilization, yield (DM, dry mass) and N content of maize, direct N_2O-N emission (annual emission and emission in three different periods), total NH_3-N emission following fertilizer application and calculated indirect N_2O emission from deposition of NH_3-N (assumption: 1% of the emitted NH_3-N is converted to N_2O-N, IPCC 2006). Values shown are means (n = 4) and standard deviations. Values (within one line) followed by the same letter are not significantly different ($p < 0.05$). Comparisons of treatments were calculated using Tukey-Test.

		Mineral fertilizer	**Digestate + NI**	**Digestate**
N fertilization				
N application rate	kg ha^{-1}	180	180	180
fertilizer type		$Ca(NO_3)_2$	digestate	digestate
Maize yield and N uptake				
total above ground biomass (DM)	t ha^{-1}	18.2 (1.8) *a*	12.7 (0.9) *b*	12.1 (0.9) *b*
grain yield (DM)	t ha^{-1}	8.7 (0.9) *a*	5.4 (0.6) *b*	5.3 (0.2) *b*
N in above ground biomass	kg ha^{-1}	212.9 (21.2) *a*	95.9 (6.6) *b*	94.0 (6.7) *b*
N in grains	kg ha^{-1}	150.6 (15.8) *a*	66.0 (6.8) *b*	65.0 (2.2) *b*
Direct N_2O-N emission				
annual emission (365 d)	g ha^{-1}	209 (66) *a*	227 (79) *a*	374 (185) *a*
grain yield related emission	g t^{-1}	24 (8) *a*	42 (15) *ab*	71 (35) *b*
Period 1 (40 d) (04/26 – 06/05)	g ha^{-1}	59 (23) *a*	62 (15) *ab*	100 (20) *c*
Period 2 (60 d) (06/06 – 08/05)	g ha^{-1}	59 (12) *a*	84 (56) *ab*	223 (164) *c*
Period 3 (265 d) (08/06 – 04/26)	g ha^{-1}	94 (40) *a*	87 (35) *a*	52 (27) *a*
NH_3 -N emission				
cumulated emissions	kg ha^{-1}	0.6 (0.3) *a*	23.5 (2.6) *b*	24.6 (3.1) *b*
indirect N_2O-N emissions	g ha^{-1}	6 (3)	235 (26)	246 (31)

3.4.2 NH_3 volatilization

Emission of NH_3 from the MIN plots was not detectable during the period after the first digestate application. After the second digestate application a small loss of 0.6 (± 0.3) kg NH_3-N ha^{-1} was measured on the MIN treatment (Figure 3-2). The treatment DIG+NI emitted 11.6 (± 1.5) and 11.9 (± 1.2) kg NH_3-N ha^{-1} following the first and second application date, respectively (Figure 3-2). From the DIG treatment 16.5 (± 2.9) kg NH_3-N ha^{-1} and 8.1 (± 0.4) kg NH_3-N ha^{-1} were volatized in April and June, respectively (Figure 3-2).

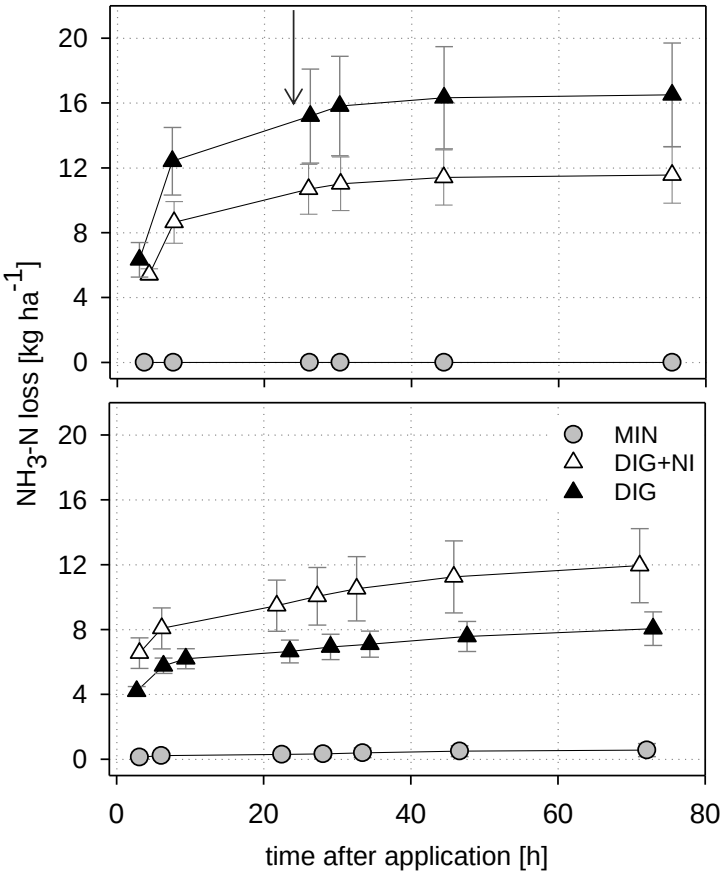

Figure 3-2: Cumulated NH_3-N losses (means of 4 field replications and standard deviations) for the different treatments following fertilization in April (upper graph) and June (lower graph) 2011. The arrow indicates digestate incorporation with a rotary harrow in April. Measurement on the MIN field plots was carried out parallel to measurements on the DIG treatments after application of the digestates, thus representing the small NH_3 -drift from the digestate amended plots.

Cumulated NH_3 emissions which include both periods of digestate application are listed in Table 3-2. Total NH_3 emission from the DIG and DIG+NI treatment did not differ (24.6 and 23.5 kg NH_3-N ha^{-1}). The overall losses from these treatments were higher in April compared to a second application in June ($p = 0.001$). In April, highest emission rates were measured on the day of application within the

first four hours, with initial fluxes of up to 1.1 kg NH_3-N ha^{-1} h^{-1}. In June, initial emission rates reached 0.9 kg NH_3-N ha^{-1} h^{-1} directly within the first hour following digestate application.

Total losses of NH_3-N accounted for 29.3% (DIG) and 27.4% (DIG+NI) of the NH_4^+-N applied with digestate. Related to the total amount of N applied, this proportion was 13.7% (DIG) and 13.1% (DIG+NI).

3.4.3 GHG balance of the investigated treatments

Figure 3-3 summarizes total GHG emission in terms of CO_2 equivalents from fertilizer production, diesel consumption for fertilizer application, direct N_2O emission and indirect N_2O emission caused by deposition of emitted NH_3-N and from the compensation of NH_3 losses by the addition of synthetic N fertilizer. Emissions related to the production of *Piadin* were not included, as no data on the CO_2 footprint were available. The total emission of CO_2 equivalents related to N fertilization increased from 536 kg CO_2-eq ha^{-1} for the DIG+NI treatment to 620 kg CO_2-eq ha^{-1} for the DIG treatment to 1832 kg CO_2-eq ha^{-1} for the MIN treatment. Highest emissions were related with the production of mineral N fertilizer ($Ca(NO_3)_2$). Emissions from production of $Ca(NO_3)_2$ were 18 times higher than direct N_2O emissions from the fertilized plots of the MIN treatment. The major part of total CO_2-eq emission from the treatments with digestate application was related to NH_3-N losses (i.e., indirect N_2O emission and compensation of NH_3-N loss by mineral N fertilizer). At our sandy site, direct N_2O emission from the fertilized field plots accounted only for 5% (MIN), 20% (DIG+NI) and 28% (DIG) of the total CO_2-eq emission. Emissions from diesel consumption were considerably higher for digestate application than $Ca(NO_3)_2$ application. However, their contribution to total atmospheric burden by CO_2-eq was < 17%.

Yield related GHG emissions calculated from maize grain yield (Table 3-2) and the total emission of CO_2-eq shown in Figure 3-3 increased in the treatment-order: DIG+NI (99 g kg^{-1}) < DIG (118 g kg^{-1}) < MIN (210 g kg^{-1}).

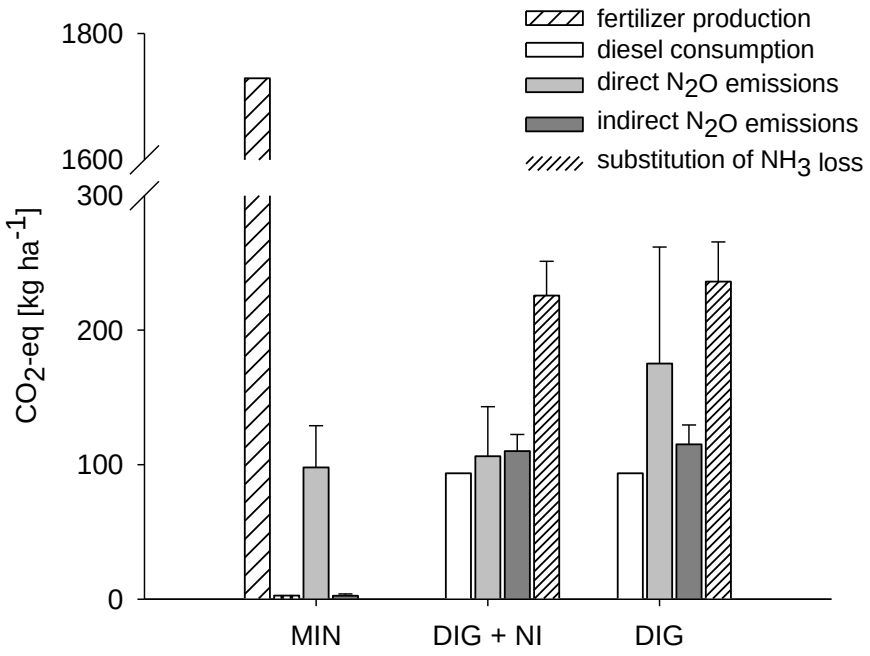

Figure 3-3: Emission of CO_2 equivalents (CO_2-eq) from different fertilization treatments and emission sources: production of calcium nitrate (fertilizer production), diesel consumption for fertilizer application (diesel consumption), direct N_2O emission, indirect N_2O emission induced by NH_3 losses and substitution of NH_3 losses by calcium nitrate (substitution of NH_3 loss). Data of direct and indirect N_2O emissions represent mean values (n = 4) and standard deviations from field measurements.

3.4.4 Soil mineral nitrogen and water filled pore space (WFPS)

All treatments showed low availability of mineral N in the upper soil (0-20 cm) from August 2011 to May 2012 (< 9.8 mg N_{min} kg^{-1} or equivalently < 30.3 kg N_{min} ha^{-1}) (Figure 3-1). However, treatments differed with respect to the availability of NO_3^- and NH_4^+ during the main growing period (May to August). The treatment with digestate and *Piadin* application (DIG+NI) showed highest contents of NH_4^+-N during *Period 1* and *2* ($p < 0.05$) (Figure 3-1). *Piadin* addition retarded nitrification and mean NH_4^+-N concentrations during *Period 1* and *Period 2* were 34.1% and 56.7% higher in the DIG+NI treatment compared to the DIG treatment. The nitrification inhibitor resulted in lower soil NO_3^- contents. Mean NO_3^--N concentrations in *Period 1* and *Period 2* were 18.0% and 20.1% lower in DIG+NI than in DIG (Figure 3-1). Soil mineral N in the MIN treatment was dominated by high NO_3^--N concentrations following fertilizer application. Nitrate availability was much higher in the MIN treatment than in the treatments fertilized with digestate.

Soil WFPS in 0 to 20 cm ranged from 16% measured in October to 77% in January (Figure 3-1). The general time course of WFPS was similar for all treatments. However from July to December, WFPS was clearly lower in the MIN treatment than in the treatments fertilized with digestate.

3.4.5 Plant yield and plant N-uptake

Total above ground maize biomass production was higher for the MIN treatment than the treatments receiving digestate (DIG, DIG+NI) (Table 3-2). Addition of the nitrification inhibitor did not influence maize biomass yield. The same relationship was found for maize grain yield which was about 60% higher for the MIN treatment than the DIG treatments (Table 3-2). Differences between MIN and the two DIG treatments were even more pronounced for the total N content in above ground biomass and grains. Maize straw remained on the field in all treatments. The N-content of the residues was 55 kg N ha^{-1} for the MIN treatment, 28 kg N ha^{-1} for the DIG+NI and 27 kg N ha^{-1} for the DIG treatment.

3.5 Discussion

3.5.1 N_2O emissions

Nitrous oxide emissions from all treatments were on a low level throughout the whole year. Slightly increased emissions occurred only in the months of fertilizer application and subsequent irrigation. The general low potential for N_2O emission was probably a result of the high sand content and the low soil moisture which hardly reached more than 60% WFPS. The latter is known as a threshold value of increasing N_2O emission by denitrification (Davidson 1991; Dobbie & Smith 2003b). Our results support the observations that soil texture is an important control factor for N_2O emission from cultivated soils and that soils with high sand content often exhibit relatively low N_2O emission rates because of the good soil aeration which limits denitrification (Bouwman et al. 2002a; Stehfest & Bouwman 2006; Weier et al. 1993). The low potential of N_2O emission was also displayed by the annual N_2O fluxes (0.21 to 0.37 kg N_2O-N ha^{-1}) which amounted to 0.12% (MIN), 0.13% (DIG+NI) and 0.21% (DIG) of the total amount of N applied with fertilizer. These values are at or even below the lower edge of fertilization-related direct N_2O-N emissions (0.18 to 15.54%) reported for Germany (Jungkunst et al. 2006). Calculation according to the IPCC guidelines (IPCC 2006), which relate N_2O emission to total N input via N fertilizers and crop residues, resulted in even lower N_2O emission factors for our treatments (0.08% to 0.18% of total N input via fertilizer and plant residues).

Effect of fertilizer type

Our results show an effect of fertilizer type on N_2O emission following fertilizer application. Nitrate application did not induce increased N_2O emission rates even if the amount of NO_3^- was strongly increased for several weeks. This shows that N_2O production was not restricted by NO_3^- availability and it supports the assumption that denitrification activity was low because of the low soil moisture and good aeration of the sandy soil. In contrast, biogas digestate resulted in increased N_2O emission

rates following application even if NO_3^- availability was much lower. Increased N_2O emission after application of organic fertilizers such as manure and slurry appears to be a general phenomenon as it was reported in several studies and for different soil conditions (e.g. Jarecki et al. 2008; Petersen 1999; Rochette et al. 2000; Velthof et al. 2003). Organic N fertilizers can affect N_2O emission in several ways depending on their N source and organic C availability. They can promote the formation and emission of N_2O via nitrification, denitrification and nitrifier denitrification. A key factor influencing N_2O emission from cattle slurry, manures and biogas digestate is probably the combination of a high content of easily mineralizable organic compounds and the availability of N. In addition, high water contents of manure and slurries may favor denitrification. Easily mineralizable organic matter can trigger denitrification and N_2O emission by different processes: as a substrate for denitrifying bacteria (Dendooven et al. 1996), as a source of mineral N for nitrification and denitrification and by its effect on metabolic activity and oxygen (O_2) consumption which can result in the formation of anaerobic microenvironments even in well aerated soils (Clemens & Huschka 2001; Comfort et al. 1990; Flessa & Beese 1995; Markfoged et al. 2011).

The effect of fertilizer type on N_2O emission has been shown to be influenced by soil moisture (Jaeger et al. 2011). Emissions have been observed to be higher for manure than nitrate application under well aerated condition whereas this effect reverses at high soil moisture and O_2 deficiency where NO_3^- addition causes the highest emissions. This effect has been attributed to the different factors restricting denitrification activity in well aerated and poorly aerated soils. In our study, the effect of fertilizer type on N_2O emission was visible only during the weeks following fertilizer application. It disappeared at the annual time scale. The results underline the importance of long-term emission monitoring for a sound assessment of N_2O emission under different fertilization strategies.

Seasonal effects on N_2O emissions (irrigation, rainfall freezing)

All fertilization treatments showed increased N_2O emission directly after irrigation which supports our conclusion that N_2O emission was primarily restricted by low soil moisture and good soil aeration and it approves that fertilizer-induced N_2O emissions are influenced by rainfall pattern after fertilization (Dobbie et al. 1999). During the investigation period, heavy rain events (> 15 mm day^{-1}) occurred four times between late August and early January and caused an temporary increase of soil WFPS to about 60 %. These rainfall events coincided with periods of lowest soil NO_3^- contents (<5 kg ha^{-1}) and did not result in increased N_2O emission activity. In contrast, irrigation, which was carried out in early summer when contents of soil mineral N were considerably higher, induced increased N_2O emission rates. The results indicate that soil NO_3^- availability was a key factor which controlled N_2O emission following water input by heavy precipitation and irrigation.

We found no evidence of increased N_2O emission during winter. High N_2O emission rates during the winter and in particular during freezing-thawing periods were reported for several sites in Germany and it was shown that these winter emissions can account for 50 to 75% of the annual N_2O flux (Dörsch et al. 2004; Flessa et al. 1995). The generally low winter emissions are probably a result of the relatively low soil water saturation even during winter (57 to 59% WFPS). As shown by Oquist et al. (2004), N_2O emissions induced by freezing-thawing cycles depend on soil moisture before freezing and the formation of a compact ice layer. Thus, the low water holding capacity of the sandy soil and the resulting low soil moisture content have probably contributed to the low winter emissions even though frost periods and freezing-thawing events occurred February. In addition, soil NO_3^- availability which also influences winter N_2O emissions (Ruser et al. 2001) was low in all treatments.

Effect of NI on N_2O emissions

The nitrification inhibitor *Piadin* reduced N_2O emission after application of digestate by 37% and 62% after the first and second application, respectively, compared to the digestate treatment without *Piadin* (DIG). Several studies which were summarized by Akiyama et al. (2010) indicate that NIs are effective in reducing N_2O emission from both chemical and organic fertilizers. Based on 85 data sets which include grassland, fields, different types of fertilizers and NIs, Akiyama et al. (2010) found a mean reduction of N_2O emission by 38%. Nitrification inhibitors potentially reduce N_2O emission by slowing nitrification, increasing nitrogen-use efficiency, and reducing denitrification (Subbarao et al. 2006). Because of the quite consistent effect of NIs on N_2O emission they concluded that NIs are a potent mitigation option for N_2O emissions (Akiyama et al. 2010).

Our results show that the mitigating effect of *Piadin* on N_2O emission disappeared on the annual time scale. The results suggest that short-term studies or studies covering only the cropping period are probably a weak basis for the general assessment of NIs as mitigation option for N_2O emission. The available information on the influence of NIs on N_2O emission is based mainly on studies which cover the vegetation period. There are only a few experiments where the effect of NIs was evaluated in experiments covering at least a whole year (Dobbie & Smith 2003a; McTaggart et al. 1997; Parkin & Hatfield 2010; Pfab et al. 2012). The results of these annual studies are not consistent. Three studies reported a reduction of total annual N_2O emission by 30 to 45 % whereas two studies (Parkin and Hatfield (2010) and our study) found only short-term effects which disappeared at the annual scale. No results are available so far on the effects of NIs on the annual N_2O emission from cropping systems fertilized with liquid manure or digestates. Several field studies covering shorter measurement periods found consistent N_2O reductions of 29 to 64% for several weeks after the use of NIs on grassland (Dittert et al. 2001; Macadam et al. 2003; Menendez et al. 2006; Merino et al. 2002; Vallejo et al. 2005) or arable soil (Meijide et al. 2007). However, long-term measurements on

the effect of NIs on N_2O emission are needed to obtain a more distinct picture of their potential to reduce total annual N_2O emission at different sites.

Maize yield and yield related N_2O emission

Yield related direct N_2O emission was primarily determined by differences in crop yield at our site. The low dry matter production and grain yield of the treatments fertilized with digestate indicates that N availability was too low to meet the maize N demand and to ensure good plant growth. The results suggest that mineralization of organic digestate N was too low to compensate for the lower content of mineral N in the applied digestate. In general, content of plant available mineral N (i.e. NH_4^+-N) is higher in biogas digestates than in most cattle or pig slurries regardless of the input substrate for digestion (solely plant based or co-digestion with animal slurry) (Fouda et al. 2013). The short-term N availability (within the year of application) of digestates depends mainly on this large NH_4^+-N pool (de Boer 2008). In our study a considerable amount of NH_4^+-N was lost by NH_3 emission which reduced the fertilizer value of the applied digestate.

Processing in biogas plants results in an increasing stability and a decreasing biodegradability of organic matter (Bernal & Kirchmann 1992; Kirchmann 1991). Mineralization of the organic N fraction of digestate is relatively slow and the contribution of this nitrogen pool to short-term fertilization effects is low (Gutser et al. 2005). However, the importance of the organic nitrogen pool as N fertilizer is increasing in systems with long-term application of organic fertilizers and thus, short- and long-term effects of fertilization with digestate have to be taken into account (Fouda et al. 2013; Gutser et al. 2005). In our study, only short–term effects were visible. The results suggest that a split fertilizer application with digestate incorporation to avoid NH_3 losses before sowing and mineral N application to the growing crop might help to compensate for slow mineralization of organic digestate N in particular in the first years of digestate application. The fertilizer treatment affected not only grain yield but also yield quality, i.e. the N content of maize grains. If direct N_2O emissions were related to total grain-N content, differences between fertilization with nitrate and digestate became even more pronounced.

The composition of the maize digestate used in this study was similar to the composition of other digestates derived from silage maize (Gericke et al. 2012; Saenger et al. 2011; Senbayram et al. 2009). According to the review of Möller and Müller (2012), our digestate from maize had a quite typical composition. We like to point out that a high utilization efficiency of digestate-N can only be expected if digestates are applied with immediate incorporation to avoid N losses via NH_3 volatilization.

3.5.2 NH_3 emissions

Biogas residues as NH_3 source

Our results confirm that biogas residues are a fertilizer with high potential for NH_3 emission (Bernal & Kirchmann 1992; Möller & Stinner 2009; Sommer et al. 2006). High NH_3 emission was attributed to the high pH value (Asmus et al. 1988; Pain et al. 1990a) and the high content of NH_4^+ in the digestates (Kirchmann & Witter 1992). Otherwise, digestates are less viscous compared to liquid manure from livestock production (Sommer & Husted 1995) and reduced viscosity can favor substrate infiltration into the soil and may consequently decrease NH_3 volatilization (Chantigny et al. 2009; Rubaek et al. 1996).

The observed NH_4^+-N related NH_3 losses (% NH_4^+-N applied) of 27 (DIG+NI) and 29% (DIG) correspond to mean relative NH_3 losses of nearly 30% measured by Wulf et al. (2002a) after trail hose application of digestate. Gericke et al. (2011) and Quakernack et al. (2012) reported 14 to 21% and 6 to 28% relative NH_3 losses from fermented renewable resources, respectively. However, besides substrate quality and agricultural management practice, NH_3 emissions strongly depend on environmental conditions, complicating direct comparison of results from different sites.

The most effective mitigation of NH_3 emissions from slurry application is fast and complete incorporation of the substrate (Malgeryd 1998; Webb et al. 2005). The high NH_3 emission in April was probably a result of the late incorporation and because the substrate was not completely removed from soil surface by the rotary harrow.

Effect of NI on NH_3 emissions

Nitrification inhibitors can increase NH_3 emission from synthetic and organic N fertilizers because of delayed nitrification and extended retention of NH_4^+-N in soil. Several studies described increased NH_3 losses if NH_4^+-containing or NH_4^+-producing fertilizers were combined with NIs (Asing et al. 2008; Cornforth & Chesney 1971; Zaman et al. 2009). We found no consistent effect of *Piadin* on NH_3 emission even if soil NH_4^+ content was highest in the digestate treatment with *Piadin*.

The meta analysis of Kim et al. (2012) on the influence of NIs on NH_3 volatilization indicates that the NIs effect depends on soil properties. Soil pH and cation exchange capacity (CEC) were found to affect not only total NH_3 emission but also the impact of NIs on NH_3 emission. Increased NH_3 losses as a result of NIs occurred in particular in alkaline soils with low CEC. Thus, the soil pH of 6.6 at our site has probably contributed to the nonexistent effect of *Piadin* on NH_3 emission.

3.5.3 Total greenhouse gas emissions

Total and grain yield related emissions of CO_2-eq were highest for the treatment fertilized with $Ca(NO_3)_2$ because of the dominance of GHG emission from fertilizer production. The large contribution of fertilizer production to total CO_2-eq emission results mainly from the low direct N_2O emission of our site. The results show that the CO_2 footprint of N fertilizer is a key factor which determines total GHG emission caused by synthetic fertilizer application. Using N fertilizer types with lower CO_2 footprint, e.g., fertilizers produced more efficiently in terms of energy and with modern emission control systems (Brentrup & Pallière 2008), can help to reduce total emissions from fertilization. The efficient substitution of synthetic N fertilizer by organic fertilizers from livestock or biogas production is another key mitigation option to avoid emissions from fertilizer production.

For the treatments with digestate application, loss of NH_3 was more important for the total GHG flux than the direct N_2O emission. The effect of NH_3 loss on atmospheric burden by CO_2-eq is due to its influence on indirect N_2O emissions and because of the lost fertilizer value. The results indicate that minimizing NH_3 losses is one of the most important measures to reduce total GHG emission from soils fertilized with digestate or other organic fertilizers with high potential of NH_3 emission. The addition of the NI had no clear effect on the yield related total emission of CO_2-eq in our experiment.

3.6 Conclusions

Direct N_2O emissions were generally low for all fertilization treatments (< 0.4 kg N_2O-N ha^{-1} yr^{-1}) which can be explained by the low water-filled pore space of the sandy soil. Emissions of N_2O were higher following digestate application than nitrate application, but at the annual scale there were no differences between fertilization treatments.

The addition of the nitrification inhibitor *Piadin* reduced N_2O emissions following digestate application by 37 to 62% but there was no effect of *Piadin* on the annual N_2O flux. Our results show that the effects of fertilizer type and *Piadin* on N_2O emission depended strongly on the length of the examined period. Thus, N_2O mitigation strategies should not be deduced from short-term measurements. In particular experiments covering the effect of nitrification inhibitors on N_2O emission from organically fertilized fields for at least a whole year are required.

Ammonia losses were the most important source of the total greenhouse gas emission from fertilization with digestate at our site. The results stress the key role of NH_3 mitigation measures to reduce greenhouse gas emission from systems fertilized with NH_3-forming fertilizers such as biogas residues or liquid manure.

Total greenhouse gas emission from fertilization with calcium nitrate was dominated by emissions related to fertilizer production. In this treatment the substitution of synthetic N fertilizer by organic

fertilizers or N_2-fixing plants and the use of fertilizer with a lower CO_2 footprint would have been efficient measures to reduce fertilization-related total greenhouse gas emissions.

Greenhouse gas mitigation strategies should be evaluated including their effect on crop yield. Our results show that the differences of yield related emissions between the tested fertilization treatments were affected and can even be reversed by the emission sources that were taken into account (e.g., direct N_2O emission, NH_3 losses, fertilizer production). All these sources of direct and indirect emissions have to be evaluated and included in strategies to reduce greenhouse gas emission from fertilizer application. We like to point out that the amount of N_2O and NH_3 emissions from fertilized fields depends strongly on site conditions (e.g., weather and soil) and management. Our results are probably characteristic for sites with low potential of direct N_2O emission.

4 Emissions of CO_2, N_2O and N_2 following maize-digestate application: separation of different carbon and nitrogen pools by isotopic labeling

4.1 Abstract

The use of digestates derived from energy crops as nitrogen fertilizer in agriculture is gaining growing importance. Due to process technology, digestates are not uniformly decomposed but consist of different organic matter pools with different degree of stability. The spreading of digestates can induce N_2O emission, but the intensity may be affected by the application technique. The effects of digestate application technique (surface application, incorporation, injection) on the dynamics of C mineralization and denitrification were analyzed in a laboratory experiment. Fresh and digested maize biomass, both enriched in ^{13}C and ^{15}N, was incubated with soil under controlled conditions to trace the pathways of emitted CO_2, N_2O and N_2. The labeling approach enabled the differentiation of two digestate C pools with different activity. The application technique had only a minor influence on mineralization of digestate C. The digestate contained a very labile organic C pool (around 20% of the total organic digestate C) which was rapidly mineralized within the first two weeks after application. The less processed C fraction of the digestate showed even higher specific C mineralization rates than incorporated fresh maize biomass. Emission of N_2O was lowest for surface applied digestate and it was similar for all of the other tested incorporation techniques. Total denitrification (N_2O+N_2) and emission of N_2O were higher following application of fresh maize biomass than digested maize. The results suggest that denitrification was primarily restricted by C availability, moreover it is indicated that the product ratio of denitrification ($N_2O/[N_2O+N_2]$) was influenced mainly by differences in soil nitrate availability. The degree of anoxic processing and the residence time in the digester are probably key factors determining the size of the more labile and more recalcitrant C fractions in digestate. Short processing of digestate triggers gaseous N losses by denitrification and may further decrease the potential for C sequestration after digestate application to soil.

4.2 Introduction

One of the current policy strategies of the European Union to tackle climate change is to promote the production of renewable energies (Renewable Energy Directive, 2009/28/EC). In Germany, especially agricultural biogas plants are widespread and the increase in biogas production is accompanied by growing amounts of fermentation residues, called digestates. Digestates are usually used as nitrogen (N) fertilizers in crop production, yet their quality largely depends on the input substrate (feedstock). Commonly used feedstocks are animal manures, waste products from food industry or agriculture and dedicated energy crops. Because of its high methane yield, (silage) maize is preferentially used in Germany. Digestates are generally characterized by a high pH value, a large proportion of ammonium (NH_4^+) on total N and reduced amounts of organic matter compared to the feedstock, but the respective contents vary among digestates from different substrates (Möller &

Müller 2012). Additionally, process technology influences digestate quality, in particular the degree of organic matter decomposition. Continuous substrate input to and removal from the fermenter leads to a digestate mixture which has experienced different retention time in the digester and is consequently not uniformly decomposed (Menardo et al. 2011).

The production of energy crops for biogas production induces changes in the soil organic carbon (SOC) stock because it includes the harvest of total aboveground biomass. There is concern that reallocation of extensively decomposed digestates cannot compensate SOC stock depletion (Möller et al. 2011). To balance soil C levels, large amounts of digestates must be applied which conflicts with the nutrient limits set by the fertilizer ordinance (Herrmann 2013). Generally, digestates may help to maintain or even increase SOC levels, because the easy mineralizable compounds were already decomposed during anaerobic digestion whereas the more recalcitrant fractions (e.g. lignin-like compounds) accumulate in the residues (Sanchez et al. 2008; Tambone et al. 2009; Tambone et al. 2010). After soil application, smaller proportions of the applied C were mineralized from digestates compared to untreated animal manures (Kirchmann & Lundvall 1993; Marcato et al. 2009), which shows their higher stability against microbial degradation.

The proportion of organic matter which is decomposed during anaerobic digestion varies between 20 to 95%, depending on feedstock composition (Möller & Müller 2012). Since substrate composition as well as the degree of degradation during anaerobic digestion have a large impact on microbial decomposition of organic material and consequently on C turnover, investigations of the different types of digestates are needed to assess their specific potential for organic matter replenishment.

Biogas production systems are often considered to have almost closed nutrient cycles, since only C is extracted during the fermentation. The plant nutrients that largely remain in the digestate can later be applied to cropping systems (Arthurson 2009). However, digestate application was frequently shown to induce considerable ammonia (NH_3) losses (Nyord et al. 2012; Möller and Stinner 2009; Amon et al. 2006), which enhance N deposition and contribute to eutrophication, acidification and indirect greenhouse gas emissions. After applying digestates to arable soil, NH_4^+-N is quickly nitrified to nitrate (NO_3^-) within a few days which increases the risk of nitrate leaching and nitrous oxide (N_2O) emissions (Möller & Stinner 2009). N_2O is a potent greenhouse gas, which is further involved in stratospheric ozone depletion and its emission may counterbalance the potential mitigation of greenhouse gas emissions associated with the use of bioenergy (Meyer-Aurich et al. 2012). In general, the application of digestates to soil can cause N_2O emission because they supply both N and available C. The reduced content of degradable organic matter and therefore lower potential for denitrification can result in lower N_2O emissions compared to undigested substrate (e.g. Chantigny et al. 2007; Petersen 1999), but was not confirmed in others studies (Bertora et al. 2008; Clemens et al. 2006). Considerable variation in N_2O emission from soils is frequently observed and can be attributed

to site-specific factors, e.g. soil texture, soil moisture, oxygen availability, temperature or application technique (Baggs & Philippot 2010; Jarecki et al. 2008; Skiba & Smith 2000; Wulf et al. 2002b) which influence N_2O emission. Albeit N_2O emissions are expected to be greater if the application technique exposes the substrate to less aerobic conditions (Webb et al. 2010), especially the effect of substrate injection remains unclear. Although several studies reported increased N_2O emission after slurry injection, (Flessa & Beese 2000; Perala et al. 2006; Wulf et al. 2002b), others found no effect of the application technique on N_2O emission (Vallejo et al. 2005; Velthof et al. 1997) or results varied with substrate type (Velthof & Mosquera 2011).

Digested energy crops represent a commonly use fertilizer but data on C mineralization and N_2O emission are scarce and it remains unclear whether information from other digestates or manure is transferable to this type of digestates.

The aim of the present work was to assess the effects of anaerobic digestion of maize biomass and of different digestate application techniques on mineralization dynamics of digestate C and gaseous N_2O and N_2 losses. Fresh and digested maize, both enriched in ^{13}C and ^{15}N was incubated in a controlled soil column incubation experiment to trace the origin of emitted CO_2, N_2O and N_2. The applied labeling approach enabled the differentiation of digestate carbon pools with different activity: an isotopically labeled fraction which was less processed and a longer processed fraction (inoculum) without labeling. Mineralization dynamics of both fractions and denitrification (emission of N_2O and N_2) was quantified simulating different application techniques: surface application, incorporation and injection with open and closed slit.

4.3 Materials and methods

4.3.1 Production of labeled biogas digestate from maize

To produce ^{13}C and ^{15}N labeled biogas digestate, maize plants (*Zea mays, L.*) were grown in buckets filled with sand in a gastight greenhouse under an atmosphere continuously enriched with $^{13}CO_2$ and fertilized with a Hoagland based nutrient solution that contained 10 atom% $^{15}NH_4{}^{15}NO_3$. Finally, harvested maize biomass had a $\delta^{13}C_{vPDB}$ value of 315.24 ‰ and a ^{15}N signature of 10.03 atom% ^{15}N.

After harvest, total aboveground plant biomass was shredded and used for the treatment with addition of ^{13}C and ^{15}N labeled fresh maize residues and, after digestion, for the treatments with addition of labeled digestate. Digestion was carried out in plastic vessels (20 l volume) containing biogas digestate from maize (80%) and barley (20%) from a commercial biogas plant to inoculate the process. The mean substrate residence time in this plant was 12 weeks. The ratio of fresh labeled maize biomass to the digested inoculum on a weight basis was 1:10.8 which was found to maintain a proper digestion process. Each vessel contained 6.5 l of the mixed substrate (130 g dry matter of

labeled maize biomass and 482 g dry matter of digested inoculum) and was diluted with 5.5 l water. The material (digested inoculum and labeled maize) was processed under anaerobic conditions at 37°C in a water quench for 7 weeks. During digestion, gas samples from the headspace (gas bags) were taken in at least weekly intervals to check the progress of digestion by analyzing the gas composition for methane (CH_4), carbon dioxide (CO_2) and hydrogen sulfide (H_2S). After digestion, the biogas digestate was stored for one week at 4°C until beginning of the experiment. Samples for analysis of digestate composition were taken after thorough stirring. In Table 4-1, the basic chemical properties are shown. The final mean isotopic signature of total N and C of the biogas digestate was 1.03 atom% ^{15}N and a $\delta^{13}C_{vPDB}$ value of 7.16 ‰.

Table 4-1: Composition of maize biomass and digestate from maize used in the incubation study.

Substrate	pH	dry matter	N_{tot}	NH_4-N	C
		%	g kg^{-1}(dry mass)	g kg^{-1}(dry mass)	g kg^{-1}(dry mass)
Digestate	7.6	3.3	98.8	64.5	393.9
Maize, fresh		45.9	41.6		958.3

4.3.2 Soil and experimental design

The soil for the experiment was collected from the 10 to 20 cm layer of an arable soil at the research farm Reinshof (51°29' N, 9°55' E, 159 m a.s.l.) located south of Göttingen, Germany. The soil type is a Haplic Luvisol derived from Loess with sand, silt and clay contents of 14%, 69% and 17%, respectively. Organic carbon and nitrogen content of the sampled soil were 12.0 and 1.2 g kg^{-1}, respectively, pH($CaCl_2$) was 6.8.

The soil was air dried, manually sieved (4 mm mesh size) and roots and other plant residues were carefully removed. The day before the onset of the experiment, soil water content was adjusted to 200 g kg^{-1} which resulted in an initial water filled pore space (WFPS) of 50%. At the start of the experiment, the soil was filled in cylindrical incubation vessels (14.4 cm inner diameter, 30 cm in height) with a filling height of 20 cm and a bulk density of 1.2 g cm^{-3}. As different digestate application techniques were simulated, bulk density was chosen from observed values of agricultural soils after field preparation. The following treatments were established with four-fold replications:

1) *Incorporation (IC):* homogeneous mixing of digestate with soil
2) *Surface application (SA*):digestate application on the surface
3) *Slit injection (SI):* digestate application in an open slit (depth: 10 cm)
4) *Closed slit injection (CSI):* similar to SI, but slit loosely covered with soil after injection
5) *Fresh maize biomass (FM)*: homogeneous mixing of fresh maize biomass with soil
6) *Control (C)*: soil without amendment

The added amount of maize digestate and fresh maize biomass was standardized to a total N application rate of 153 kg ha^{-1} (64 mg added N kg^{-1} dry soil). In terms of organic carbon, 254 mg C kg^{-1} dry soil were applied with the digestate, and 1121.6 mg C kg^{-1} dry soil were added to the treatment with fresh maize biomass. The fresh maize material (whole plants, above ground harvest) was shredded directly after harvest and kept at -20°C before use.

Three additional empty vessels (blanks) were included in the incubation system to measure background concentrations of the incubation atmosphere. A second set of 32 incubation vessels was prepared to allow destructive soil sampling for extraction of mineral nitrogen after three weeks.

4.3.3 Incubation experiment and gas sampling

The incubation experiment was carried out for 76 days at 16°C in darkness in a climate chamber using a soil incubation system as described by Hantschel et al. (1994). After preparation of the treatments, the incubation vessels were airtight sealed and the headspace of each incubation vessel was continuously flushed with 10 ml min^{-1} of a synthetic air. The synthetic air was produced from pure pressurized gases using a gas mixer (HovaGAS digital G8-vTI, IAS GmbH, Frankfurt, Germany). It consisted mainly of helium with a mixture of 20% O_2, 5% N_2 and 319 ppb N_2O. The proportion of N_2 was kept low to increase the detection limit for N_2 evolved from the ^{15}N labeled substrate (Meyer et al. 2010), which was digestate from labeled maize and labeled fresh maize biomass in our case. The mixed synthetic air was humidified before entering the incubation vessels by conducting it through water-filled glass vials to avoid soil water losses by evaporation. This resulted in constant water content during the 76 days of incubation with a mean evaporation loss < 2 g kg^{-1} soil.

Gas samples were collected daily from each vessel during the first two weeks of incubation. During the following 6 weeks sampling frequency was reduced to two samples per week, ending up with one sampling per week for the last 3 weeks of the experiment. According to Well et al. (2006), septum capped glass vials for gas samples were permanently flushed with air from the headspace outlet of the incubated soil columns. For $^{15}N_2$ and ($^{15}N_2$+$^{15}N_2O$) and $^{13}CO_2$ analysis, additional gas samples were taken during the first 8 and 15 days, respectively.

4.3.4 Gas analyses

Concentrations of N_2O and CO_2 were measured with gas chromatography. Samples with N_2O concentrations > 1 ppm (i.e. during the first week of experiment) were measured on a gas chromatograph with an electron capture detector (ECD) (Agilent 7890A, Santa Clara, USA). The precision of the gas analysis expressed as the coefficient of variation for repeated determinations of standard gases was < 1 % for CO_2 and N_2O. Gas samples with CO_2 and N_2O concentrations below 2000 and 1 ppm, respectively, were measured with a gas chromatograph (GC 2014, Shimadzu,

Duisburg, Germany) equipped with an ECD detector and an automated rack (P 65, Loftfields Analytical Solutions, Neu Eichenberg, Germany). The precision of CO_2 and N_2O analysis was checked weekly by repeated determination of standard gases (380 ppm CO_2, 320 ppb N_2O) and was consistently < 3 %.

The ^{15}N abundance in N_2O and N_2 was determined using the technique described in detail by Lewicka-Szczebak et al. (2013). Briefly, this method is based on sample analysis with a modified GasBench II (Thermo Scientific, Bremen, Germany) coupled to an isotope ratio mass spectrometer (IRMS, MAT 253, Thermo Scientific, Bremen, Germany). In this setup, stable isotope ratios $^{29}N_2/^{28}N_2$ (R29) and $^{30}N_2/^{28}N_2$ (R30) in N_2 are measured and N_2O is optionally reduced to N_2 which allows to determine the isotope ratios for N_2, N_2O or the sum of N_2+N_2O in one sample. However, the ^{15}N labeling applied in our experiment was too low to detect changes in R30. Therefore, further calculations were based on the R29 measurements only. This approach still allowed the precise determination of the fraction of N_2 and N_2O originating from the N pool of the ^{15}N labeled substrate (Spott et al. 2006, section 5.3.5), but did not allow the simultaneous independent determination of the actual ^{15}N atom fraction in the total N pool in amended soils emitting N_2 and N_2O. N_2O concentration derived from the ^{15}N labeled substrate pool was either directly obtained from R29 of the N_2O peak of the chromatogram or indirectly from the difference of the N_2 and N_2+N_2O peaks. The detection limit for N_2+N_2O derived from digestate or maize was around 0.42 ppm.

The ^{13}C abundance in CO_2 was measured with an isotopic ratio mass spectrometer (Delta plus, Thermo Scientific, Bremen, Germany) equipped with a Combi-Pal Autosampler (CTC-Analytics, Zwingen, Switzerland) at the Centre for Stable Isotope Research and Analysis (KOSI, University of Göttingen, Germany). The ^{13}C abundance of the samples given as delta values (δ), and is defined as:
$\delta^{13}C\ (‰) = (R_{sample}/R_{standard} - 1)*1000$, where R = 13C/12C.

4.3.5 Source partitioning of gas fluxes

N_2 and N_2O fluxes from the ^{15}N labeled substrate

The treatments studied in this experiment enabled quantification of N_2O+N_2 fluxes derived from the labeled maize pool. However, estimation of N_2O+N_2 fluxes from soil N pool was not possible, since we have not established a treatment with ^{15}N-labeled soil. Moreover, the digestate consisted of two N pools, the ^{15}N labeled maize and the unlabeled N pool of digestate inoculums. Hence, N_2 and N_2O fluxes from this latter pool were also not detected. The following evaluations therefore refer explicitly to the fractions of N_2O, N_2, or N_2O+N_2 derived from the labeled maize pool, which had an initial ^{15}N abundance of 10.03 atom%. Calculations were based on a two-pool model using the non-

random distribution approach (Spott et al. 2006). The fractions of N_2 or N_2O in the gas sample derived from N added with the labeled maize substrate (f_{label}) is defined as

$$f_{label} = \frac{N_x(pool)}{N_x(total)} \quad (1)$$

where $N_x(pool)$ is N_2, N_2O or N_2+N_2O derived from the labeled pool, and $N_x(total)$ is the total concentration of these species in the gas sample. f_{label} was calculated according to equation (4) from Spott et al. (2006), independently from R30:

$$f_{label} = \frac{1}{1-\frac{R29(1-a_2)^2-2a_2(1-a_2)}{R29(1-a_1)^2-2a_1(1-a_1)}} \quad (2)$$

where *a2* is the ^{15}N abundance of pool 2 and *a1* is the ^{15}N abundance of pool 1. f_{label} was thus calculated from R29 of the N_2, N_2O or N_2+N_2O peaks. Since we estimated N emitted from the labeled maize added directly to the soil or to the digestate, a_2 was the ^{15}N abundance of the labeled maize, i.e. 10.03 atom%. a_1 is the ^{15}N abundance of the incubation atmosphere, which corresponds to atmospheric N_2 (0.366 atom%).

The concentration (ppm) of labeled-maize-derived N in N_2 (afterwards referred to as: N_2m) or N_2+N_2O (N_2m+N_2Om) in the headspace of the incubation vessel was finally calculated rearranging Eq. 1 to:

$$N_x(pool) = f_{label} \times N_x(total) \quad (3)$$

where $N_x(total)$ was obtained from N_2 and N_2O concentrations from GC analysis.

Concentrations of N_2Om in the headspace were calculated by subtracting the calculated N_2m concentrations from the total N_2m+N_2Om concentrations.

The detection limit was calculated as follows:

$$r_{sa} - r_{bg} > 3 \times sd\, r_{bg} \quad (4)$$

where r_{sa} and r_{bg} are the 29/28 N_2 ratios in sample and background atmosphere (taken from the incubation vessel without soil for each sampling date), respectively and sd r_{bg} is the standard deviation of repeated r_{bg} measurements on each sampling date.

Finally, fluxes of N_2O-N in total and of N_2m-N and (N_2m+N_2Om)-N were calculated as follows:

$$F = \frac{a*w}{m} \quad (5)$$

where *F* is the N_2O or N_2 flux (µg h^{-1} (kg dry soil)$^{-1}$), *a* is the concentration of captured N_2O or N_2 (µg l^{-1}), *w* is the flow rate of incubation atmosphere through the vessel (l min^{-1}) and *m* is the dry mass of the incubated soil (kg).

The cumulated N_2O-N emissions for 76 days were calculated by summing up all daily fluxes for the first 10 days of the experiment and afterwards by linear interpolation between the nearest sampling dates. Cumulated emissions originating from the labeled maize pool (N_2m and N_2m+N_2Om) were calculated for the first 8 days of the experiment. As described above, mean fluxes were summed up or linearly interpolated between two nearest sampling dates. Due to the occurrence of very low N_2m and (N_2m+N_2Om) fluxes (below detection limit) several gaps within the datasets existed. To enable calculation of cumulated Nm-fluxes, gaps were filled as follows:

if (1) N_2m was missing: *0.5 x detection limit (N_2m*) for the respective measurement date. If (2) (N_2m+N_2Om) was additionally missing: *0.5 x detection limit (N_2m+N_2Om)* and N_2m was subsequently calculated from *0.5 x detection limit (N_2m+N_2Om) x mean product ratio ($N_2Om/[N_2Om+N_2m]$)*.

CO_2 fluxes from the ^{13}C labeled substrate

Due to the mixture and combined digestion of ^{13}C labeled fresh maize biomass and non labeled digested inoculum-substrate, the finally applied digestate consisted of two differently decomposed C pools with distinct $\delta^{13}C$ signatures. After digestate application, a third C pool i.e. the soil-borne organic C pool was relevant. Assuming that no priming effect due to the addition of digestate occurred, the digestate-derived CO_2 emissions from soil were calculated. Therefore, the $\delta^{13}C$ signature of emitted CO_2 was corrected for dilution by non labeled CO_2 from the control treatment. The further partitioning of digestate-derived CO_2 into labeled-maize-derived CO_2 (CO_2m) and inoculum-derived CO_2 was calculated considering the $\delta^{13}C$ values of the maize, the inoculum and the CO_2 produced by digestate mineralization. The $\delta^{13}C$ value of the CO_2 from digestate mineralization (δ_{min}) was calculated as follows:

$$\delta_{min} = \frac{(\delta_{sample} * c_{sample}) - (\delta_{control} * c_{control})}{(c_{sample} - c_{control})} \quad (6)$$

where $\delta_{sample/control}$ is the $\delta^{13}C$ value (CO_2) of the sample/control treatment and *c* is the concentration of CO_2 (ppm) of the sample/control treatment.

The δ_{min} consists of two fractions that sum to a whole, fraction (f_1) for source 1 (δ_{maize} = 315.2 ‰) and fraction (f_2) for source 2 ($\delta_{inoculum}$ = -17.4 ‰):

$$\delta_{min} = (\delta_{maize} * f_1) + (\delta_{inoculum} * f_2) \quad (7a)$$

The share of the fraction f_1 is calculated according to:

$$f_1 = (\delta_{min} - \delta_{inoculum}) / (\delta_{maize} - \delta_{inoculum}) \quad (7b)$$

Similar to the calculation of N_2O-N emissions, total and labeled-maize-derived CO_2-C efflux was calculated by using equation 5. The cumulative losses were calculated by summing up all daily CO_2-C

fluxes for the whole experiment and assuming that, where no daily data were available, the daily CO_2-C fluxes changed linearly between the nearest sampling dates for which samples were available.

4.3.6 Calculation and modeling of specific carbon mineralization rates

Specific daily C mineralization rates (mg g^{-1}) were calculated for the different substrate pools (i.e. soil derived organic C, labeled-maize-derived digestate C, non labeled digestate inoculum C, fresh labeled maize biomass C) from the ratio (daily CO_2-C flux)/(C pool size). We took into account that the size of these different organic carbon pools decreased with time depending on the observed specific mineralization rate.

The dynamics of the specific C mineralization rates of the different substrates (i.e. soil derived organic C, digestate C, fresh labeled maize biomass C) during the incubation period of 75 days were fitted using a two-pool single exponential decay model:

$$M(t) = c_0 + c_1{}^{(-k*t)} \quad (8)$$

where c_0 is the long term mineralization rate (mg g^{-1}) (slowly mineralizing, recalcitrant carbon pool), c_1 is the initial mineralization rate (mg g^{-1}) (quickly mineralizing carbon pool) and k is the mineralization rate constant ($1d^{-1}$). The models were fitted using the Gauss-Newton algorithm in the software R (3.0.2, The R Foundation for Statistical Computing 2013). In order to test for differences between treatments, nested models (i.e. two separate models vs. a combined model) were compared with the likelihood ratio test. Resulting p-values were adjusted for multiple testing with the Bonferroni correction. Effects were considered significant for $p < 0.05$.

The dynamics of the specific mineralization of the two digestate C pools (i.e. originating from the labeled-maize and the digested inoculums) was determined for the first 15 days of the experiment. Consequently, a mineralization rate for the more stable and thus slower mineralizing C pool (c_0) could not be estimated. Therefore, data of C mineralization of these two digestate C pools were fitted to a single exponential decay model.

4.3.7 Statistical analyses of cumulated gas fluxes

Mean values of the treatment replications and standard deviations were calculated for the cumulated production of N_2O, N_2m, (N_2m+N_2Om), CO_2 and CO_2m. Datasets of N_2O and CO_2 were calculated for the whole 76 days of the experiment, for N_2m and N_2m+N_2Om for the first 8 days and for CO_2m for 15 days after the start of the experiment. Differences in cumulative emissions were tested with analysis of variance (ANOVA), followed by pairwise comparisons between group levels (t-tests) with an adjustment method that corrections for multiple testing. We used the *fdr* method

(Benjamini & Hochberg 1995; Benjamini & Yekutieli 2001) since it is more powerful to detect possible differences between treatment groups compared to other correction methods (e.g. Bonferroni). Effects were considered significant for $p < 0.05$. Statistical analyses were performed using the software R (3.0.2, The R Foundation for Statistical Computing 2013).

4.3.8 Soil mineral nitrogen

Soil mineral nitrogen (NO_3^- + NH_4^+) was sampled three times during the experiment. At the first day of the experiment, mineral N was determined in the control soil and directly after addition of the substrate in FM and IC treatments. Three weeks after the start of the experiment, the parallel incubated soil columns were used for extraction of mineral N. At the last day of the experiment the soil cores that were prior used for gas sampling were extracted. Nitrate and NH_4^+ were extracted from thoroughly homogenized soil samples with 0.01 $CaCl_2$ and a soil to solution ratio of 1:4 (w/v). After filtration (MN614 ¼ filters, Macherey & Nagel, Düren, Germany) the extracts were stored at -20°C until analysis of mineral N concentrations with a continuous flow analyzer (SA 5000, Skalar Analytical B.V., Netherlands). For the treatments with digestate injection, the injection slit was sampled separated from the remaining soil core. For that, a defined volume around the injection slit including soil up to approx. 20 mm distance from the slit surface was taken. Subsamples were weighted to determine the relative contribution of the slit-samples to the total soil core. Afterwards, mean soil mineral N contents were calculated for all treatments to allow comparison of mineral N dynamics in the entire incubation column.

4.4 Results

4.4.1 CO_2 emissions

Emission rates of CO_2 of the control treatment remained on a relatively stable level (~120 µg CO_2-C kg^{-1} h^{-1}) throughout the investigation period (Figure 4-1). For the different digestate application treatments, CO_2-C emission rates were strongly increased during the first days of incubation with initial values of 300-500 µg CO_2-C kg^{-1} h^{-1}. After two weeks emissions from all digestate treatments reached a level which was close to the respiration rate of the control treatment. The treatment with addition of fresh maize biomass (FM), which received the highest input of organic C, also showed a consistent decrease of CO_2 emission over time, though emissions started on a higher level (1600 µg CO_2-C kg^{-1} h^{-1}) and persisted longer compared to the digestate treatments.

During the initial 15 days of the experiment, 23% of total CO_2-C emitted during the incubation period of 76 days was produced in the control treatment and among the digestate treatments between 32% and 35%. Nearly half (46%) of total cumulated CO_2-C emissions were lost during the first 15 days

from the FM treatment. Subtracting the background emission of the control treatment, the total emission of CO_2-C after 15 days was 19% to 21% of the C applied with the digestate and 28% of the C applied with the fresh maize. The total CO_2-C release in the control treatment after 15 days was equivalent to 0.5% of the total soil organic C content (Table 4-2).

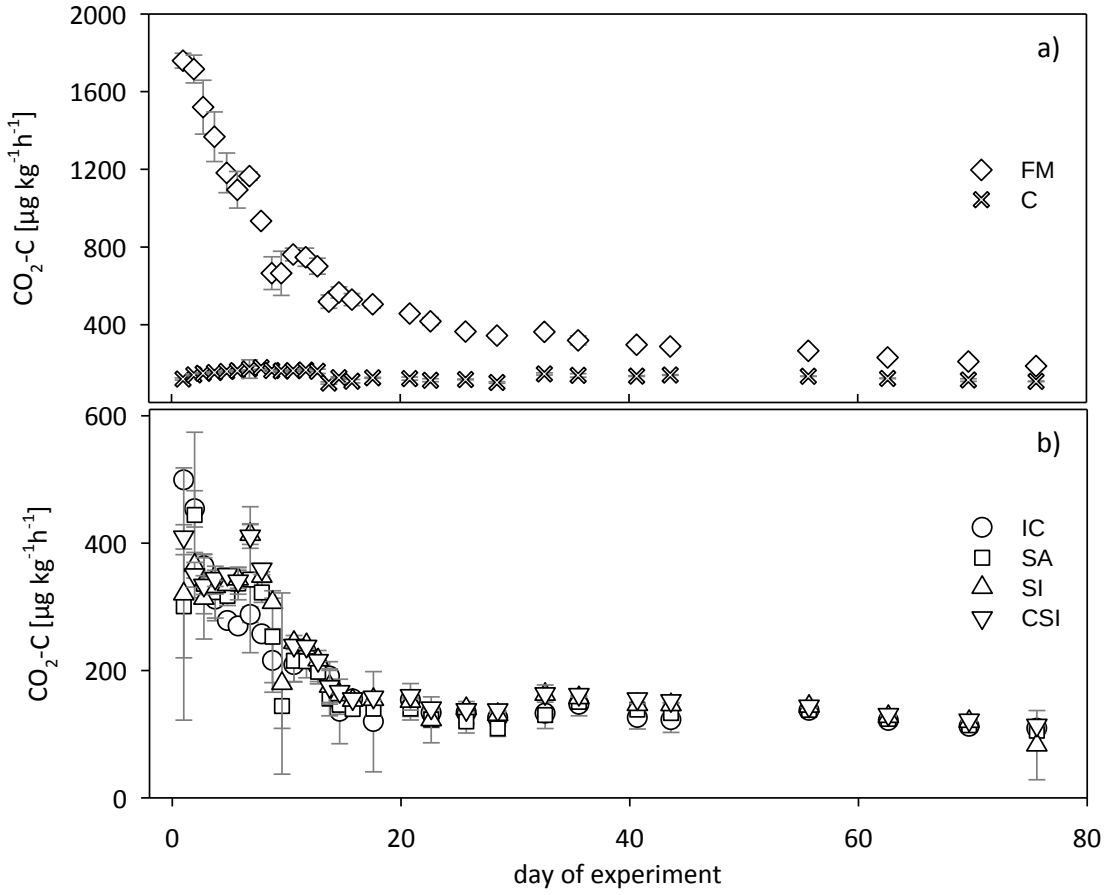

Figure 4-1: Time course of CO_2-C emission rates per kg dry soil (n = 4, standard deviations from the treatment replications) from the differently treated soil columns during the incubation of 76 days. Treatments upper graph (a): FM: incorporated fresh maize biomass, C: soil control. Treatments lower graph (b): IC: digestate incorporation, SA: surface application of digestate, SI: slit injection of digestate, CSI: closed slit injection.

The amount of applied C which evolved as CO_2-C within 76 days was 27% to 33% from the different digestate application treatments and more than 50 % from the treatment with fresh maize biomass. In the control treatment, 2 % of the soil organic carbon was lost as CO_2-C emission during the whole experiment (Table 4-2). For both 15 and 76 days, cumulated CO_2-C emissions were significantly higher ($p < 0.05$) for the treatments amended with digestate or fresh maize compared to the soil control. The FM treatment emitted further more CO_2-C than the digestate treatments ($p < 0.001$) (Table 4-2). The addition of digestate increased cumulated CO_2-C emissions by 28 to 35%, incorporation of fresh maize by 237% compared to the control treatment. We found no effect of the digestate application technique on the total CO_2 emission after 15 and 76 days (Table 4-2).

Table 4-2: Mean cumulated CO_2-C losses from the differently treated soil columns after 15 and 76 days (n = 4, standard deviations from the treatment replications). CO_2m-C losses represent the emissions derived from the ^{13}C labeled maize pool (only for 15-days-period). Proportions of added C and CO_2m-C were calculated by subtracting the background emission of the soil control. Different letters indicate significant differences between treatment means ($p < 0.05$). Treatments: IC: digestate incorporation, SA: surface application of digestate, SI: slit injection of digestate, CSI: closed slit injection, FM: incorporated fresh maize biomass, C: soil control.

	15 days			**76** days	
Treatment	**∑ CO_2-C** mg kg^{-1}	**% CO_2m-C on total ∑ CO_2-C**	**% of added C**	**∑ CO_2-C** mg kg^{-1}	**% of added C**
IC	102.7 ± 5.6 *a*	22.1	18.6	325.1 ± 28.6 *a*	33.2
SA	106.8 ± 11.0 *a*	23.1	20.2	316.3 ± 26.3 *a*	29.8
SI	109.5 ± 5.7 *a*	25.5	21.3	315.7 ± 19.4 *a*	29.5
CSI	106.6 ± 5.7 *a*	28.1	20.1	308.9 ± 15.5 *a*	26.9
FM	368.5 ± 24.3 *b*	101.2	27.9	809.7 ± 43.0 *b*	50.7
C	55.4 ± 1.8 *c*	-	0.5*	240.6 ± 7.3 *c*	2.0*

* % soil organic carbon emitted as CO_2

4.4.2 Specific mineralization rates of different carbon pools

The dynamics of the specific mineralization rate (expressed as mg CO_2-C emitted per gram substrate carbon) of different organic C pools (digestate, fresh maize, soil organic carbon) during the incubation of 76 days is shown in Figure 4-2. Initial specific mineralization rates of digestate and fresh maize biomass were similar with about 30 mg $g^{-1}d^{-1}$. The increasing stability of the remaining C pools and the resulting decreasing specific mineralization rates with time could be described well by the fitted two pools mineralization models.

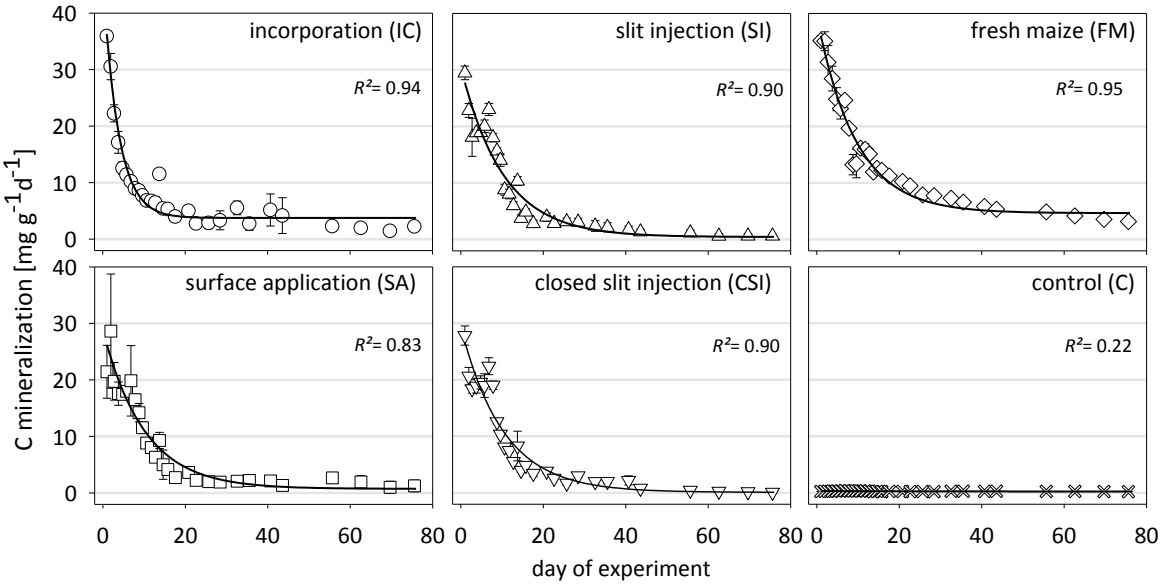

Figure 4-2: Dynamics of measured and modeled (black line) specific C mineralization rates (mg CO_2-C per g substrate C) of the different substrates (digestate, fresh maize biomass) or of soil organic carbon (control treatement) in differently treated soil columns (n = 4 and standard deviation) The R^2-value indicates the quality of the fit.

Modeled mineralization dynamics of the C pool of the fresh maize biomass treatment was different from the digestate treatments, irrespective of application type ($p < 0.0001$, Table 4-3). The two pool models showed a higher specific mineralization rate of the fast decomposing C pool for the uniform incorporation of digestate (IC treatment, $p < 0.0001$) than for the other techniques of digestate application (SA, SI, CSI) (Table 4-3).

Table 4-3: Parameters of the first-order kinetic model used to describe C mineralization from the applied digestate or maize biomass in the differently treated soil columns (soil organic C in the control treatment). During the first 15 days, mineralization of digestate C was further distinguished between the carbon originating from the less processed ^{13}C labeled maize (pool derived from labeled maize) and the longer processed not labeled C pool within the digestate. Different letters indicate significant differences between C mineralization models in total (not only for *k*-values) ($p < 0.05$). Treatments: IC: digestate incorporation, SA: surface application of digestate, SI: slit injection of digestate, CSI: closed slit injection, FM: incorporated fresh maize biomass, C: soil control.

	Non labeled pool (15 days)		Pool derived from labeled maize (15 days)		Total digestate (76 days)		
Treatment	c_1	k	c_1	k	c_0	c_1	k
IC	29.86	0.16 *a*	199.23	0.24 *a*	3.76	42.66	0.27 *a*
SA	20.75	0.08 *b*	127.88	0.10 *b*	0.73	28.12	0.10 *b*
SI	21.39	0.08 *b*	138.83	0.07 *b*	0.44	30.11	0.10 *b*
CSI	19.46	0.08 *b*	145.57	0.07 *b*	0.10	29.27	0.10 *b*
FM	-	-	-	-	4.67	34.88	0.10 *c*
C	-	-	-	-	0.24	0.08	0.04 *d*

c_1: organic C mineralization rate (mg g^{-1}) (quickly mineralizing C pool), k: mineralization rate constant (d^{-1}), c_0: long term C mineralization rate (mg g^{-1}), only for 76-day-period

The digestion of ^{13}C labeled maize biomass allowed the separation of two different C pools within the applied digestate: the less processed digestate originating from the ^{13}C labeled maize biomass and the more processed not labeled digested inoculum. Figure 4-3 shows the time course of the measured $\delta^{13}C$ signature of CO_2 emitted from the differently treated soil columns. CO_2 released from the control showed a typical natural $\delta^{13}C$ signature for soils under C_3 vegetation ($\delta^{13}C_{vPDB}$: -24.4 ‰). Treatments with digestate application emitted CO_2 enriched in ^{13}C reflecting the changing contribution of labeled digestate to soil respiration (Figure 4-3).

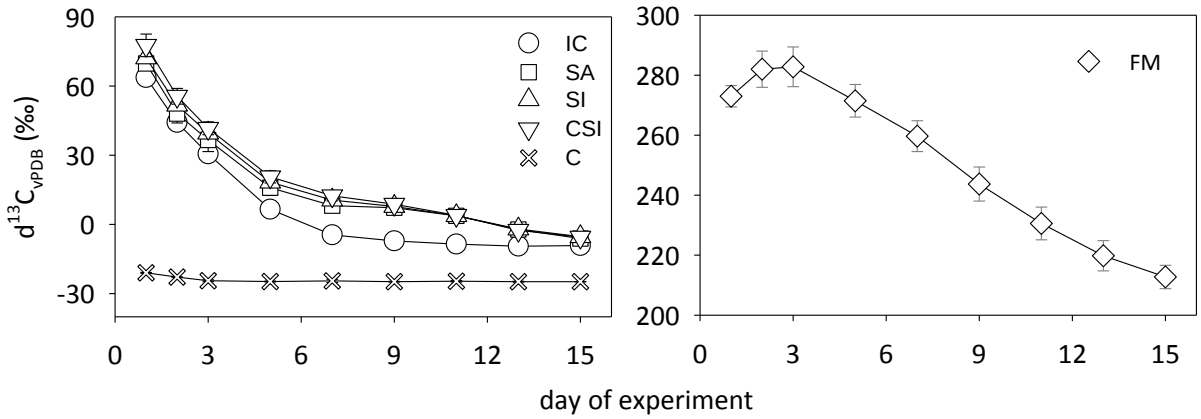

Figure 4-3: Time course of mean $\delta^{13}C_{vPDB}$ values of emitted CO_2-C (n = 4, with standard deviation) from differently treated soil columns. Treatments: IC: digestate incorporation, SA: surface application of digestate, SI: slit injection of digestate, CSI: closed slit injection, FM: incorporated fresh maize residues, C: soil control.

Highest $\delta^{13}C_{vPDB}$ values of up to 282.8 ‰ were measured in CO_2 emitted from the soil columns with fresh labeled maize biomass. Standard deviation of the $\delta^{13}C$ signature of CO_2 emitted from replicated soil columns was generally small and thus only partly visible in Figure 4-3. The isotopic signatures of CO_2 emitted from treatments with digestate application were used for CO_2 source partitioning during the first 15 days of incubation. The cumulated CO_2 emission for the first 15 days (Table 4-1) showed that between 22% (IC) and 28% (CSI) of the cumulated CO_2-C were derived from the labeled maize in the digestate.

Calculated specific mineralization rates were several times higher for the less processed digestate originating from labeled maize than for the digested inoculum (Figure 4-4). The single C pool mineralization models for the data of the less processed ^{13}C labeled digestate C had a coefficient of determination (R^2_a-values) between 0.78 (CSI) and 0.96 (IC) which confirms that the regression model describes the data well (Figure 4-4). The treatment with homogenous digestate incorporation (IC) mineralized faster than the other treatments ($p < 0.001$) for both the labeled and the non labeled carbon pool (Table 4-2).

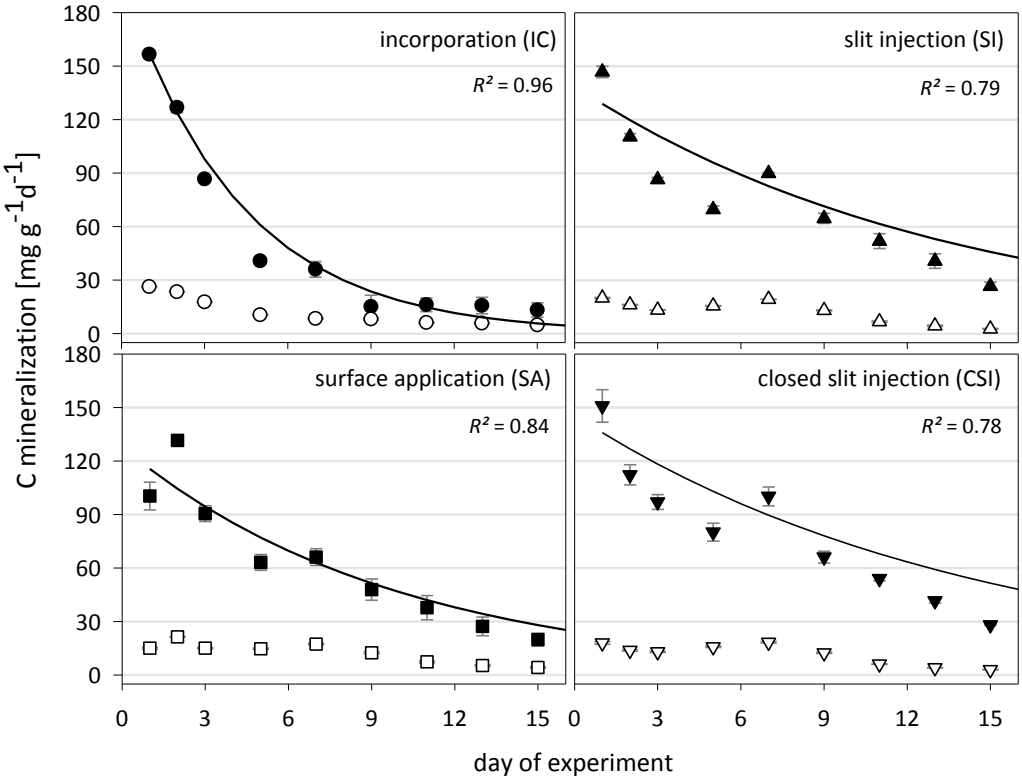

Figure 4-4: Calculated mean specific mineralization rates (mg CO_2-C per g substrate C) of the less processed digestate originating from the ^{13}C labeled maize biomass (black symbols) and the more processed not labeled digestated inoculum (white symbols) during the first 15 days of the experiment (n = 4 and standard deviation). The black line is the modeled ^{13}C mineralization rate, the R^2-value indicates the quality of the fit.

4.4.3 Priming effects

The produced CO_2 from the treatment with fresh maize biomass (FM) originated from mineralization of the fresh plant material and from decomposition of soil organic matter. We calculated the contribution of these two organic C pools to the measured CO_2-C emission from the $\delta^{13}C$ signatures of the two substrate pools (δ_{maize} = 315.24 ‰, δ_{soil} = -27.07 ‰) and the emitted CO_2-C using Eq. 7a and 7b. We compared the CO_2-C emission from mineralization of soil organic C in the two treatments FM and control (C) to identify possible priming effects induced by the addition of the maize biomass during the first 15 days of incubation. Total cumulative CO_2-C emission from mineralization of soil organic C was 55.4 ± 1.8 mg kg^{-1} and 50.5 ± 4.2 mg kg^{-1} (mean and standard deviation) for the control and the treatment FM, respectively. There was no significant difference ($p > 0.05$). Comparing mineralization rates of soil organic C at single sampling dates, we found no clear consistent difference between the two treatments. Mineralization rates were not different at 3 of the 9

sampling dates (day 2, 13 and 15), at day 1 mineralization rates were higher and at days 3 to 11 it was lower for FM than C.

4.4.4 Nitrogen dynamics

Total N_2O emission

Highest mean N_2O-N emission rates of up to 27.7 µg kg^{-1} h^{-1} were measured on the first day of the experiment from the soil columns with incorporation of fresh maize biomass (FM) (Figure 4-5). Emissions dropped down on the following day to 2.60 µg kg^{-1} h^{-1} and steadily decreased over time. All treatments where digestate was placed in the soil (IC, SI, CSI) showed highest N_2O emission on the first day after application (Figure 4-5). During the following days the digestate incorporation (IC) treatment showed the same constant decrease as observed for the fresh maize incorporation treatment. Both injection treatments (SI, CSI) showed a prolonged N_2O emission activity with a second emission maximum after 6 to 8 days. Afterward emissions declined and reached a low level which was close to the emission rate from the control treatment. After 16 days of the experiment, the peak of N_2O-N fluxes from the digestate amended treatments had passed and emission rates adapted to the level of the control. The emission rate from the control treatment (C) was consistently around 0.03 µg $kg^{-1}h^{-1}$. The treatment with surface application of digestate (SA) showed a different behavior. It did not show an initial emission peak and exhibited constantly low N_2O emission rates (< 1 µg $kg^{-1}h^{-1}$).

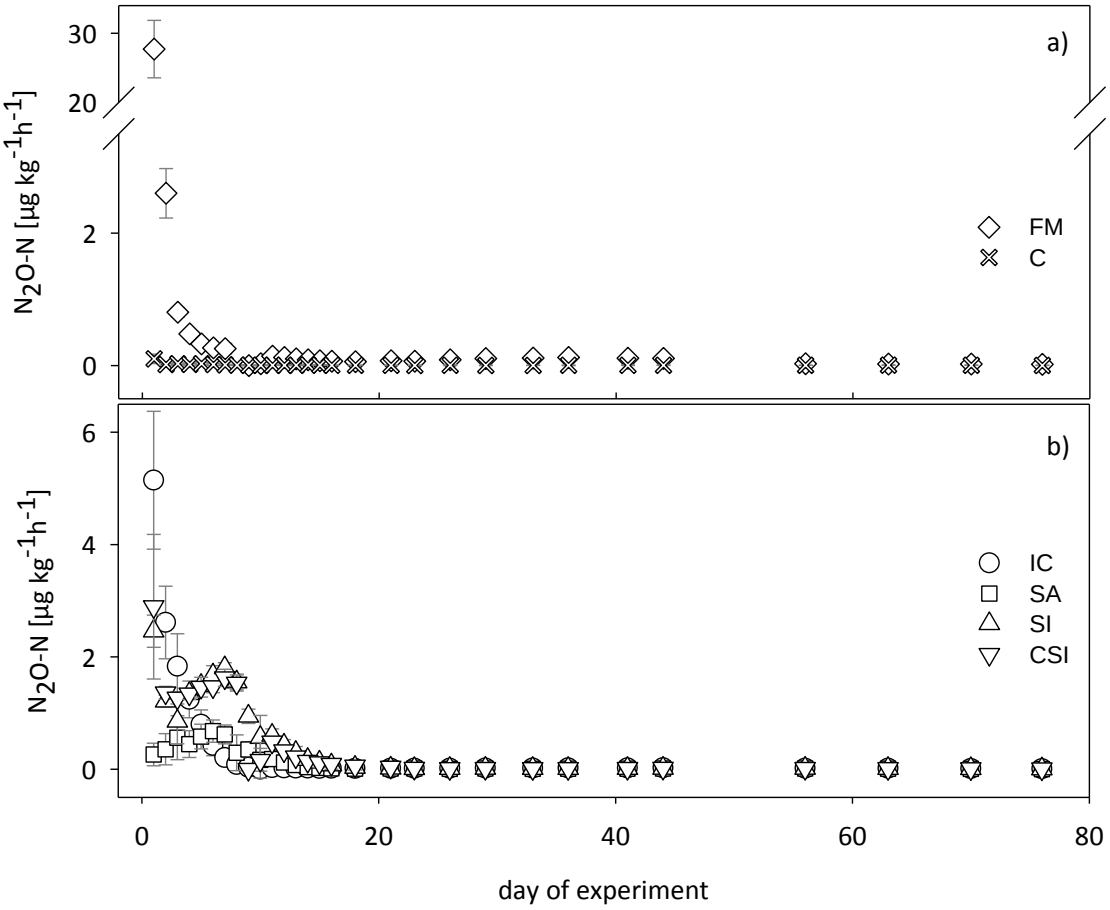

Figure 4-5: Time course of N_2O-N emission rates (n = 4, standard deviations from the treatment replications) from the differently treated soil columns during the incubation of 76 days. Treatments upper graph (a): FM: incorporated fresh maize biomass, C: soil control. Treatments lower graph (b): IC: digestate incorporation, SA: surface application of digestate, SI: slit injection of digestate, CSI: closed slit injection.

Cumulated mean N_2O-N emissions for each treatment calculated for 8 and 76 days are shown in Table 4-4. Summed mean N_2O-N emissions for 8 and 76 days were significantly smaller from the control treatment compared to all other treatments ($p < 0.01$). The FM treatment emitted more N_2O-N ($p < 0.001$) than the digestate treatments. In addition, for both periods, the digestate SA treatment emitted less N_2O compared to the other digestate application treatments ($p < 0.01$). Different digestate incorporation techniques (IC, SI, CSI) had no effect on cumulative N_2O fluxes. After 76 days, the total N_2O-N emission were 0.2% (SA) and 0.5% (IC, SI, CSI) of the added digestate N and 1.6% of the fresh maize N added. After 8 days, between 72% (SA) and 91% (IC) of the total measured N_2O-N emissions were already evolved from the fertilized treatments and 44% from the soil control.

Table 4-4: Mean cumulated gaseous N losses per kg soil from the differently treated soil columns after 8 and 76 days (n = 4, standard deviations from the treatment replications). N_2m-N and N_2Om-N losses represent the emissions derived from the labeled maize pool (initial ^{15}N abundance: 10 atom% of maize-N) (only for 8 days period). Different letters indicate significant differences between treatment means ($p < 0.05$). Treatments: IC: digestate incorporation, SA: surface application of digestate, SI: slit injection of digestate, CSI: closed slit injection, FM: incorporated fresh maize biomass, C: soil control.

	8 days			**76** days
Treatment	**∑ N_2O-N**	**∑ N_2m-N**	**∑ N_2Om-N**	**∑ N_2O-N**
	µg kg^{-1}	µg kg^{-1}	µg kg^{-1}	µg kg^{-1}
IC	296.3 ± 78.7 *a*	41.9 ± 19.1 *a*	17.3 ± 4.0 *a*	324.0 ± 81.9 *a*
SA	97.6 ± 41.2 *b*	10.9 ± 6.0 *a*	10.0 ± 3.4 *b*	135.6 ± 51.9 *b*
SI	297.5 ± 23.7 *a*	16.7 ± 3.1 *a*	23.7 ± 2.9 *a*	395.9 ± 39.4 *a*
CSI	311.0 ± 47.3 *a*	22.3 ± 5.3 *a*	23.8 ±4.4 *a*	374.2 ± 51.6 *a*
FM	766.7 ± 122.9 *c*	6986.0 ± 746.1 *b*	176.8 ± 223.1 *c*	875.9 ± 127.5 *c*
C	5.3 ± 1.7 *d*	-	-	12.1 ± 2.1 *d*

4.4.5 Emission of N_2O and N_2 from the labeled substrate

Except for the first sampling day, the time course of (N_2m+N_2Om)-N and N_2m-N emissions of the FM treatment (Figure 4-6a, b) were similar. Total emission activity in terms of (N_2m+N_2Om)-N peaked on the 4th day and it declined afterwards. The calculated N_2Om-N/(N_2m+N_2Om)-N product ratio of denitrification sharply dropped from nearly 0.4 to close to zero after the first day (Figure 4-6c). All digestate treatments showed the lowest (N_2m+N_2Om)-N emission activity at the end of the 12 days incubation period. The dynamics of emissions during the first days differed among the different digestate treatments. Until day 5, the incorporation treatment (IC) showed higher (N_2m+N_2Om)-N emissions than the other digestate treatments. Total emission (N_2m+N_2Om)-N from labeled injected digestates were highest after 7 days, whereas the surface application (SA) treatment emitted only little (N_2m+N_2Om)-N. The product ratio (N_2Om-N/(N_2m+N_2Om)-N) was highest for both injection treatments from day 5 to 8, ranging between 0.4 and 0.6. During the first 8 days, the mean proportion of N_2Om on total N_2Om+N_2m emissions was 29% for the IC treatment, 48% for SA and 59% and 52% for SI and CSI, respectively. In the FM treatment, only 3% of total N_2Om+N_2m were emitted as N_2Om. For both N_2Om-N and N_2m-N, the FM treatment emitted significantly more ($p < 0.001$) compared to the digestate treatments (Table 4-4).

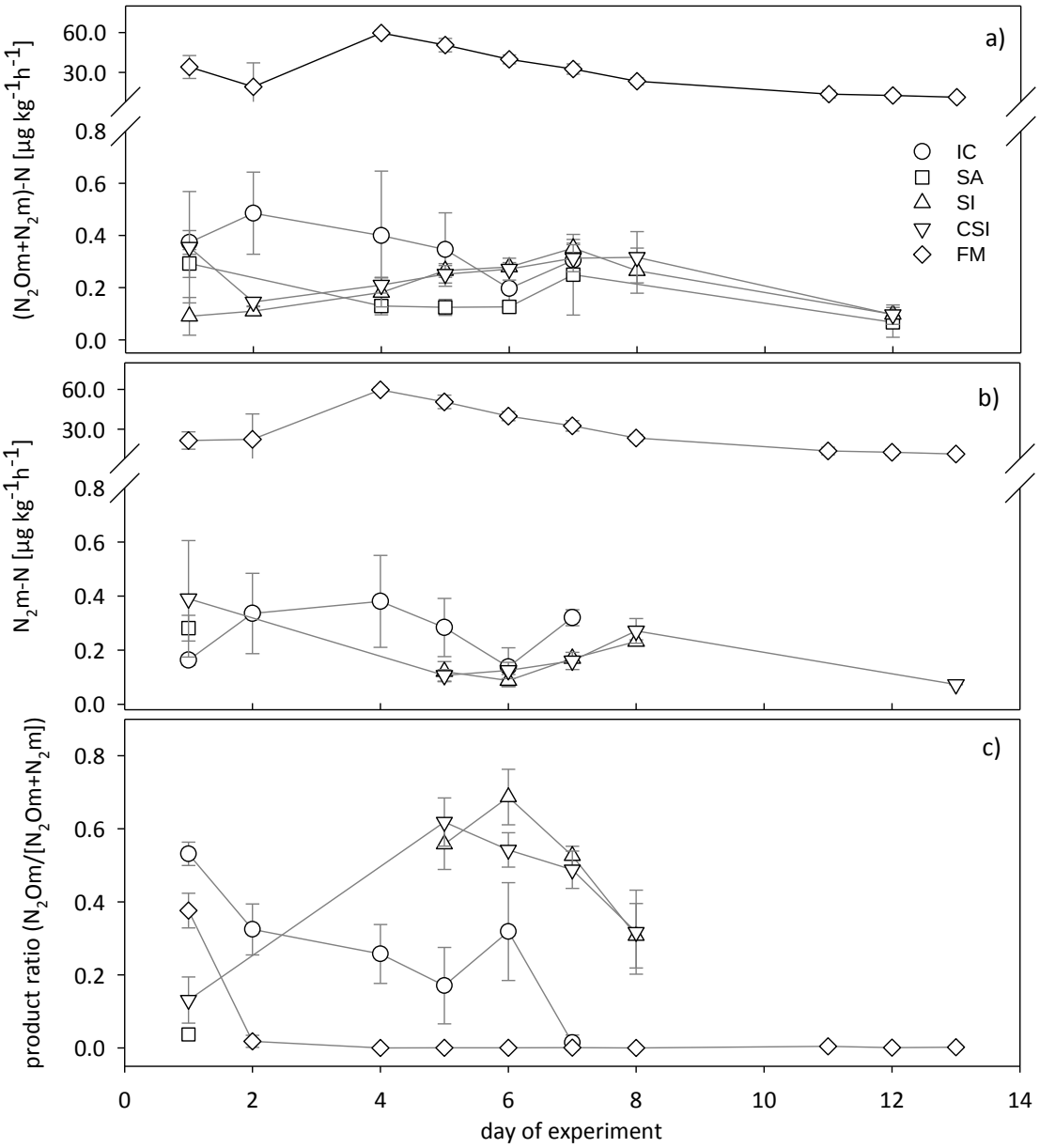

Figure 4-6: Mean (N_2O+N_2)-N (a) and N_2-N (b) emission rates derived from the labeled maize pool (initial ^{15}N abundance: 10 atom% of maize-N) for the different treatments during the first 13 days of the experiments and their product ratio (c). Data is shown as mean values (n = 4) with standard deviations. Treatments: IC: digestate incorporation, SA: surface application of digestate, SI: slit injection of digestate, CSI: closed slit injection, FM: incorporated fresh maize biomass.

The share of N_2Om on total N_2O emissions during the first 8 days was only between 6% (IC) and 10% (SA). Denitrification losses ([N_2Om+N_2m]-N) related to the applied labeled maize-N were 0.5% (SA), 0.9% (SI), 1.0% (CSI) and 1.3% (IC) for the digestate treatments, respectively and 14.7% for the treatment with fresh maize biomass. For N_2Om-N emission, these proportions were 0.2% (SA), 0.4% (IC, FM) and 0.5% (SI, CSI), respectively.

4.4.6 Soil mineral nitrogen

Significant amounts of NH_4^+-N were only found in the treatments with digestate application at the first sampling date (Figure 4-7). Soil mineral N contents were dominated by NO_3^-. Except for the FM treatment, NO_3^--concentrations increased throughout the incubation period (Figure 4-7). The initial soil NO_3^--N background concentration was 11 mg kg^{-1} dry soil for all treatments prior to the experiment. The addition of biogas digestate resulted in an increase of the mean soil NO_3^- content of 50 to 70 mg kg^{-1} soil within 76 days. Final mean NO_3^--concentrations were 36, 63, 76, 80 and 81 mg NO_3^--N kg^{-1} dry soil in C, IC, SA, SI and CSI, respectively. A small decrease of the soil NO_3^- content of about 10 mg (kg soil)$^{-1}$ was found in the FM treatment on the first sampling date. By the end of the experiment, this treatment showed also an accumulation of nitrate with 39 mg NO_3^--N kg (dry soil)$^{-1}$ (Figure 4-7).

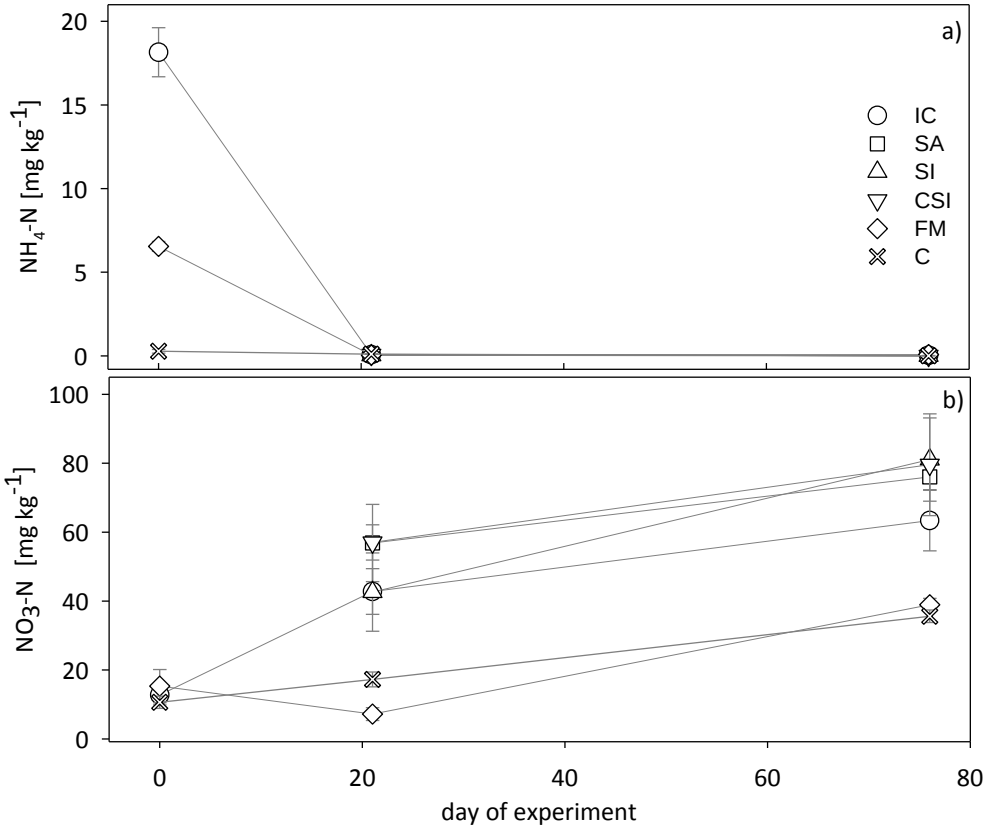

Figure 4-7: Concentration of soil mineral nitrogen (upper graph: NH_4^+, lower graph: NO_3^-) at the beginning of the experiment, after 21 days and in the end. Data is shown as mean concentrations for the complete incubation vessels (n = 4, standard deviations from treatment replications). Treatments: IC: digestate incorporation, SA: surface application of digestate, SI: slit injection of digestate, CSI: closed slit injection, FM: incorporated fresh maize biomass, C: soil control.

At the first sampling date, mean NO_3^- concentrations extracted from the injection slit were 2.4 (SI) and 2.9-fold (CSI) increased compared to the surrounding soil and no NH_4^+ was detectable. This ratio decreased to 1.3- (SI) and 1.7-fold (CSI) higher mean NO_3^- concentrations in the slit at the final sampling.

4.5 Discussion

4.5.1 Carbon dynamics

CO_2 emission and specific mineralization rates of different carbon pools

Stability of organic C in digestate is a key factor determining the potential of digestate for soil C sequestration and a sustainable management of soil organic C in rotations with biogas crops. Our results provide information on the dynamics of digestate C mineralization, the total mineralization within 11 weeks, and the existence of pools with different specific mineralization rates.

In all treatments, the application of digestates led to an increase in CO_2 emission compared to the control treatment. This emission dynamics is in line with previous studies on soil CO_2 fluxes after digestate (e.g.Jaeger et al. 2013; Koester et al. 2011; Saenger et al. 2010) or cattle slurry application (Fangueiro et al. 2010; Flessa & Beese 2000). Generally, the increase in microbial activity as indicated by CO_2 production is related to the additional supply of easily degradable organic material. The higher respiration activity indicates an enhanced microbial degradation of organic substance. Although C availability is reduced due to anaerobic digestion, the application of digestate was shown to increase soil respiration and soil microbial biomass, albeit much smaller compared to plant residues (Chen et al. 2012; Johansen et al. 2013). Our results show that 27 to 33 % of the digestate carbon was emitted as CO_2 within 76 days. Carbon mineralization of digestate was similar to that observed for digestates in earlier incubation experiments with arable soils: 28 to 38% of total digestate C during 53 days (Jaeger et al. 2013) and 34% during 42 days (Saenger et al. 2011) for maize digestates, 30% during 56 days for co-digested cattle slurry and maize-oat silage (de la Fuente et al. 2013) and 30 to 34% during 56 days for stable digestates from cattle or pig slurry with agro-industrial wastes (Alburquerque et al. 2012). Likewise, Terhoeven-Urselmans et al. (2009) reported a total mineralization of 27% of the digestate derived C during 50 days of their field experiment. In an incubation experiment, Chen et al. (2012) measured five times lower C mineralization (in terms of CO_2 emissions) of biogas digestate compared to fresh maize residues and attributed this to the high resistance of digestates to microbial degradation caused by higher lignin contents in the remaining organic matter of digestates. Similar results were obtained by Thomsen et al. (2013) who found a two-fold increase in CO_2 emissions from fodder (mainly consisting of silage maize) compared to the

digested substrate which supposedly reduced soil microbial activity after application. Marcato et al. (2009) showed in their soil incubation study, that digested pig slurry was more stable with respect to decomposition than untreated pig slurry. However, compared with farmyard manure, compost or aerobically stored animal manure, digestates were showed higher C mineralization following soil application (Grigatti et al. 2011; Jaeger et al. 2013; Kirchmann 1991).

The higher stability of digestates compared to animal manure or crop residues were attributed to the enrichment of more stable organic compounds (e.g. lignin-like material, lipids) (Marcato et al. 2009; Tambone et al. 2009; Tambone et al. 2010) which are known to be humus precursors (Lorenz et al. 2007). Consequently, digestates have a higher potential for soil organic matter replenishment than undigested material and may be used as organic amendment.

Mineralization of organic substrates largely depends on the substrate type which varies between and also within the same animal species, e.g. due to different substrate treatment or animal diet (Morvan et al. 2006). Likewise, also the composition of digestates can vary widely, depending on the type and properties (e.g. organic matter content) of feedstock (e.g. energy crops, animal manure) and technical operating parameters of the digestion plant. The organic loading rate in combination with the hydraulic retention time in the digester were shown to influence the digestibility (methane yield) of the residual organic matter (Menardo et al. 2011). Longer hydraulic retention times result in higher proportion of fibrous material (Menardo et al. 2011) which is less degradable after application to soil (Tambone et al. 2009). To evaluate the organic matter stability of digestates, a comprehensive assessment of different input substrates and process technologies is required. Up to now it remains unclear, whether single results of digestate C stability are applicable to digestates derived from different feedstock.

The application technique had no effect on total mineralization of digestate C. We have not found another study on the effect of different digestate application techniques on mineralization dynamics, however our results agree with earlier findings of Flessa and Beese (2000) or Dendooven et al. (1998) from different slurry application treatments which indicated no effect of the application technique on mineralization of slurry C. Even if total mineralization of digestate C was not influenced by the application technique we found a distinct effect on the mineralization dynamics. The two pool modeling approach indicated that the initial C mineralization rate of the quickly decomposing C pool was higher for the treatment with homogenous digestate incorporation than for the other digestate application treatments. This enhanced microbial turnover may be a result of a fast aeration of the digestate and a better substrate to soil contact which can affect decomposition dynamics (Coppens et al. 2006). Close contact with the soil microflora is a key factor for the degradation of the substrate (Henriksen & Breland 2002).

The dynamics of the specific mineralization rates revealed that digestate contained highly bioavailable organic C but also considerable amounts of quite stable organic compounds. During the digestion process, easily available organic C is decomposed whilst volatile fatty acids (VFAs) are produced as intermediate products which are further decomposed to CO_2 or methane (CH_4) within the digester. Following the exposure to aerobic conditions in soil, VFAs are metabolized within a few days (Kirchmann & Lundvall 1993; Paul & Beauchamp 1989) and probably provoked the initial respiration peak.

The continuous substrate input in biogas plants produces biogas residues which experienced different residence times in the digester. The mean residence time describes only the average time of processing in the digester, however, the biogas residues generally represent a mixture of more and less processed substrate. The decomposition experiment with labeled digestate provided an insight into the variability of the mineralization rates of more and less processed digestate constituent. Bioavailability and mineralization rates of the labeled, less processed digestate were much higher than mean mineralization of digestate C. The initial specific CO_2 emission from these less processed biogas residues was even much higher than the initial CO_2 release from decomposition of fresh maize biomass. The results show the importance of extended substrate treatment for stabilization of the remaining organic matter in digestate. The reduction of this labile organic C fraction may also affect gaseous N losses induced by digestate application since denitrification is favored by easily available organic C and the associated increased microbial O_2 consumption.

The initial high rates of decomposition of the labeled digestate C, which was independent of the application technique, reflect the presence of readily available C at this point. The decreasing mineralization rates at later stages and in more processed digestate are probably a result of lower contents of VFAs and a greater resistance to decomposition of the lignin-cellulose complexes (Levi-Minzi et al. 1990). The results indicate that the value of digestate for soil C sequestration increases with extended substrate processing during anaerobic digestion because this yields a digestate with enhanced resistance to microbial decomposition under oxic conditions in soils.

Priming effect

The priming effect is defined as a short-term change in the turnover of soil organic matter by the treatments (usually addition of organic C) of the soil (Kuzyakov et al. 2000). Since soil organic matter turnover is not directly measured, the priming effect is often related to changes in CO_2 efflux rates with extra CO_2 production due to priming. We evaluated the priming effect through comparison of the soil organic C turnover in the fresh maize biomass treatment and the control treatment and did not find higher total soil CO_2-C emission rates from the FM treatment, and even lower emissions

rates for most dates. In their review study, Blagodatskaya and Kuzyakov (2008) evaluated earlier works on priming effects and found a relationship between the induced extra efflux of CO_2 (priming effect) and the amount of added organic C in terms of soil microbial biomass C. In the present study, the amount of added organic C was nearly 400% of soil microbial biomass in the FM treatment (data not shown) and according to Blagodatskaya and Kuzyakov (2008), priming effects tended to be close to zero or even negative in the range of 200-500% substrate addition. As expected, we did not find a positive priming effect but mostly lower soil organic C mineralization in the FM treatment. For the digestate treatments, C addition in terms of soil microbial biomass C was around 100% (data not shown). For this range, only a small priming effect is expected through the addition of easily available substrates (glucose, amino acids) (Blagodatskaya & Kuzyakov 2008). However, in particular the amount of sugars was shown to be significantly reduced during anaerobic digestion (Provenzano et al. 2014). Therefore we also expected no priming effect from the digestate treatments. Consequently, the calculation of digestate-derived CO_2 fluxes by the subtraction of soil CO_2 fluxes (control treatment) from total CO_2 fluxes in the digestate treatments was appropriate and did not overestimate C mineralization.

4.5.2 Nitrogen dynamics

Dynamics of N_2O emission

Time patterns of N_2O emissions showed that N_2O emission increased directly after substrate application. This emission dynamic is often observed after the application of organic fertilizers such as animal slurries, manure or digestates (e.g. Flessa & Beese 2000; Velthof et al. 2003). The supply of easily degradable organic material leads to increased microbial respiration with an enhanced O_2 demand by the fast-growing microbial community (Petersen et al. 1996). This in turn creates an O_2 depleted environment or causes anoxic microsites which favor denitrification (Clemens & Huschka 2001; Dendooven et al. 1998). This process actually happens within a few hours (Stevens & Laughlin 2001).

In the FM treatment, the high initial N_2O losses are attributed to denitrification. This assumption is further confirmed by the dynamics of the $N_2O/(N_2O+N_2)$ product ratio in the FM treatment: denitrification losses occurred in the form of N_2, the end product of the denitrification. Our results indicate that the risk of high initial N_2O losses increase with the amount of easily degradable organic C in the added substrate (i.e. highest emission from the treatment with largest input of easily decomposable organic matter). The higher initial N_2O emission rates from the treatments with homogenous substrate incorporation further indicates that reduction of soil nitrate is supported by a large interface of soil and the available C source. Rapid aeration and small interfaces with soil nitrate

have probably restricted N_2O emission from surface applied digestate. In contrast digestate injection resulted in prolonged N_2O emission activity. These observations are in line with findings from Thomsen et al. (2010), who reported prolonged N_2O emissions from field injection of digested pig slurry compared to surface application. The N_2O production rates at a later time in our study can be attributed to concomitant nitrification and denitrification processes, since the addition of digestate or plant residues simultaneously provides degradable organic C and N. Findings of Koester et al. (2011) confirm this assumption in a study with ^{15}N labeled biogas digestate as they showed that denitrification was initially the dominating process but was shifted towards nitrification after depletion of the labile organic C pool.

In total, N_2O emission accounted for 0.2 to 0.5% of the N applied with the digestates, which is a smaller proportion than reported by Koester et al. (2011) (0.9% of digestate N), Saenger et al. (2010) (1.4 to 1.7%), or Senbayram et al. (2009) (2.6%). In the incubation study of Jaeger et al. (2013), 24% of added digestate N were emitted as N_2O. Large variation in N_2O emissions from different soil types and with regard to differences in application technique, substrate quality, temperature or soil water content is well known. The low proportion of N_2O emitted in our study in contrast to the other results may be due to the lower soil WFPS, which was at 50% in the present study but varied between 60 and 80% in the other experiments. An increase in soil WFPS is known to stimulate N_2O production and highest emissions occur at WFPS levels between 60 and 90% (Dobbie & Smith 2003b).

Emission of N_2O and N_2 from the labeled substrate

The simultaneous measurement of N_2 and N_2O from the labeled maize pool allowed identifying different emission dynamics among the digestate application treatments. All treatments with substrate addition showed significant emissions of N_2Om-N and N_2m-N. This indicates a coupled nitrification-denitrification of substrate N in hot-spots around organic fertilizer particles (Nielsen et al. 1996). Generally, high denitrification activity is expected after the application of organic fertilizers to soil, especially during the initial period (Senbayram et al. 2009) and also under well drained conditions (McKenney et al. 1993).

Denitrification rates of labeled substrate N, expressed as the sum of emitted N_2Om-N and N_2m-N, were highest in the treatment with incorporation of fresh maize biomass (FM). Except for the first incubation day, denitrification of maize N in this treatment was dominated by production of N_2.

The product ratio of denitrification ($N_2O/[N_2O+N_2]$) was shown to be affected by the ratio of NO_3^- and available organic C in arable soils (Weier et al. 1993). Senbayram et al. (2012) reported that in presence of high NO_3^- concentrations, a higher $N_2O/(N_2O+N_2)$ product ratio was measured because NO_3^- had an inhibitory effect on N_2O reduction. Maximum product ratios varied between soil types,

but in all soils, denitrification N_2O product ratios increased significantly with higher NO_3^- concentration (Senbayram et al. 2012). Our treatment with incorporation of fresh maize biomass showed the greatest availability of organic C (displayed by the highest CO_2 emission rates) and it had the lowest soil NO_3^- content in particular at the beginning of the experiment when soil NO_3^- fell below the concentration found in the control treatment. This high reduction potential at low soil NO_3^- content might explain the observation that N_2 was the main end product of denitrification in this treatment. All treatments with digestate application showed lower C availability and much higher contents of NO_3^- and exhibited a more balanced product ratio of denitrification (N_2Om-N/[N_2Om+N_2m]-N: 0.1 to 0.7) with greater proportions of N_2O.

Total denitrification of labeled substrate N was similar in all treatments with digestate incorporation but the dynamics of denitrification activity and of the ratio of N_2Om-N to N_2m differed. Highest product ratio of N_2Om-N/(N_2Om+N_2m)-N developed in the treatments with digestate injection after about 6 days when most of the easily available C was mineralized and nitrification of the large digestate NH_4^+-pool resulted in strongly increasing NO_3^- contents. The results suggest that the combination of the accumulation of large amounts NH_4^+ in the slit zone which can be rapidly nitrified after aeration and the rapidly decreasing amount of easily mineralizable organic C in digestate promote emission of N_2O by denitrification. A similar dynamics of denitrification losses was reported from Comfort et al. (1990), where concurrent nitrification-denitrification of slurry inorganic N first favored N_2O emission and N_2 emission occurred later after depletion of NO_3^- in the injection zone until decreasing organic C and soil moisture content. An option to reduce denitrification and N_2O emission from digestate injection might be the temporal separation of the periods of high C mineralization and NO_3^- production by the addition of a nitrification inhibitor. After slurry injection, nitrification inhibitors were shown to efficiently reduce denitrification losses in field and incubation studies for several days (deKlein et al. 1996; Dittert et al. 2001), thus including the period of main N_2O and CO_2 emissions (Comfort et al. 1990).

The low emission of labeled digestate N from surface applied digestate is in line with the general low denitrification activity of this treatment. It supports the conclusion that fast aeration at the soil surface restricts denitrification.

Effect of digestate application technique

Surface application of the digestate (SA) resulted in smallest N_2O-N losses for both 8 and 76 days, no differences existed among the other digestate treatments. This result confirms earlier findings from Flessa and Beese (2000) that injection of cattle slurry leads to higher N_2O losses compared to surface application. However, this effect of application technique was not consistently observed in previous incubation studies (e.g. Dendooven et al. 1998; Velthof et al. 2003). Due to the relatively high soil

WFPS of 67% which induced favorable conditions for denitrification, Flessa and Beese (2000) concluded that the high water content had contributed to the distinct application technique effect in their study. Generally, the injection of liquid manure or slurry creates wet O_2-depleted zones which foster local denitrifying sites since the C and N rich injection slit is temporally stable (Markfoged et al. 2011; Petersen et al. 2003). The results in the literature and of the present study indicate that the application type may affect N_2O emissions. However, there is no consistent and overall effect of application technique on N_2O emission because various factors and their diverse interactions are known to control N_2O emissions, e.g. soil type, soil moisture content and availability of O_2, abundance of organic matter and mineral nitrogen or contact between substrate particles and soil. In particular for biogas digestates, studies comparing different application techniques are lacking. Emissions from digestate application might be similar to emissions from animal slurries. However, there are some changes during digestion which hamper direct comparability such as NH_4^+-N content, organic C availability, dry matter content or viscosity. Further verification is needed to what extent results from different application techniques of pig and cattle slurry can be directly applied to biogas digestates.

For the use of fertilizers in agriculture, avoiding N_2O losses is important. However, selection of the appropriate application technique should not only focus on reducing direct N_2O emission since digestates are also important sources of NH_3 (e.g. Möller & Stinner 2009; Thomsen et al. 2010). Possible trade-offs of N_2O and NH_3 emissions have to be taken into account. Losses of NH_3-N can be much larger than emissions of N_2O-N. They reduce the value of digestate as N fertilizer and induce indirect N_2O emissions. Due to difficulties in measuring NH_3 emissions from closed soil cores with a low air exchange rate, no data on NH_3 losses are available in the present study. But in particular for biogas digestate it was previously shown that after surface application, NH_3 emission can dominate the total greenhouse gas emission in terms of CO_2 equivalents (e.g. Wulf et al. 2002b).

Soil mineral nitrogen dynamics

Soil mineral nitrogen dynamics clearly differed between the FM treatment and the digestate treatments. In FM, soil NH_4^+-N content was generally low and there was a net depletion of soil NH_4^+ and soil NO_3^- during the first 20 days of incubation. Frequently observed, such initial decrease in soil mineral N can be explained by microbial N immobilization in particular during periods with high decomposition activity (Alburquerque et al. 2012; Bernal & Kirchmann 1992; Kirchmann & Lundvall 1993). In addition, denitrification activity has contributed to the decreasing NO_3^- contents (decrease of 10 mg NO_3^--N kg^{-1} soil during the first 20 days) in our experiment, which was indicated by concomitant N_2m-N emissions of 7 mg kg^{-1} from the maize pool during the first week. The fast nitrification of NH_4^+-N and associated NO_3^- accumulation in the digestate treatments is a commonly

observed pattern after biogas residue application (Abubaker et al. 2013; de la Fuente et al. 2013; Dorno et al. 2013; Koester et al. 2011).

Soil NO_3^--N in the injection affected soil was around two times higher than observed in the entire soil, which can be attributed to the concentration of digestate NH_4^+ in and around the injection slit (Petersen 1992). The high NO_3^- contents in digestate treatments indicate that denitrification activity in these treatments was clearly not restricted by NO_3^- availability. However, NO_3^- availability may have affected N_2O emission and the denitrification product ratio ($N_2O/[N_2O+N_2]$). Senbayram et al. (2009) concluded for biogas digestates that they likely promote high N_2O losses when the ratio of NO_3^-/available C increases after nitrification and reaches a certain threshold value, but only if soil moisture was high. This is in line with earlier work by Weier et al. (1993), concluding that the final reduction step of denitrification is controlled by NO_3^- availability. These findings are in agreement with the differences in soil NO_3^- content in our treatments and the observed product ratio of denitrification.

Although results from laboratory studies can only be transferred to field-scale to a limited extent, the quick nitrification of digestate NH_4^+ should be implicitly considered when digestates are applied as fertilizers. The risk of NO_3^- leaching is high if digestates are spread too early or in too large doses to meet the plant's N demand. The use of a nitrification inhibitor could help to delay nitrification and stabilize the NH_4^+ content in soil.

4.6 Conclusions

Digestate C was shown to be less mineralized compared to the fresh maize biomass (30% versus 50% CO_2-C loss from the added substrate), with only a minor influence of the application technique. However, the results indicated that the applied digestate contained an organic fraction (about 20-28% of the digestate C) which is highly labile under oxic conditions in arable soils. This C fraction was rapidly mineralized within two weeks and resulted in high soil respiration rates. In particular the less processed C fraction within the digestate showed even a much higher specific mineralization rate than incorporated fresh maize biomass. The size of this labile C fraction affects the value of digestate for soil carbon sequestration and it appeared to play an important role for denitrification and N_2O emission. The results indicate that the less processed organic matter contributes preferentially to the labile C pool in the digestate. Therefore, the degree of anoxic processing and the residence time in the fermenter are probably key factors determining the size of the more labile and more recalcitrant C fractions in digestate.

Surface application of digestate resulted in lower N_2O emission than differently incorporated substrate. However, surface application of digestate without immediate incorporation is not recommended because of high NH_3 emissions.

The results indicate that denitrification and N_2O emission was primarily influenced by C availability which promotes the formation of microhabitats with oxygen deficiency. Simultaneous measurement of N_2O and N_2 suggest that the product ratio of denitrification (($N_2O/[N_2O+N_2]$) was influenced mainly by differences in soil nitrate availability.

Particularly with regard to their high proportion of plant-available nitrogen, digestates are a valuable fertilizer. Nevertheless, digestion and timing of digestate application have to be adapted in a way that this fertilizer value is optimized, soil C sequestration is supported and gaseous and dissolved N losses are minimized. Composition and properties of digestates can vary considerably depending on the digested substrates and the digestion process. This hampers the generalization of results on N and C dynamics and it suggests that specific evaluations are necessary for digestates of different origin.

5 Synthesis and general conclusions

Digestates can be used as N fertilizer in crop production and should be recycled in the agricultural system to close nutrient cycles and to avoid nutrient losses. The varying content of nutrients depending on input substrate requires a thorough analysis of the digestate composition to assess its value as fertilizer. Different types of N losses can considerably reduce the value of digestate as N fertilizer and reject the concept of closed nutrient cycles. A main challenge for the farmer is therefore to avoid these losses in order to benefit from digestates as valuable N fertilizer and to reduce pollution of the atmosphere and the environment.

In this thesis, digestates, particularly from maize, were studied with respect to their potential for NH_3 and N_2O emissions after field application and their effect on C mineralization. The combined evaluation of the three investigations can give a more comprehensive understanding of the processes regulating N and C turnover following digestate application to soil.

5.1 Importance and mitigation of NH_3 emission following digestate application

Digestates from energy crops are a new, growing source of NH_3 emission which is not yet taken into account in the national German inventory of NH_3 emissions. Accounting for these emissions will significantly increase total NH_3 emission from Germany. Strategies to minimize NH_3 emissions from digestates are urgently needed to keep this increase as small as possible. The additional emissions counteract the political aim to reduce NH_3 emission. The results from the field studies underline the high potential of digestates for NH_3 volatilization. Emission from digestates was higher compared to non-digested pig and cattle manure and solid-liquid separation was not efficient in reducing NH_3 emissions in total (Chapter 2). Solid fractions emitted even more NH_3 related to their NH_4^+ content compared to non-separated substrates. Solid fractions of digestates are not comparable to other solid organic amendments, e.g. farmyard manure and compost which experienced decomposition under oxic conditions. The accumulation of NH_4^+ even in solid digestate fractions results in a higher risk of NH_3 losses. Hence, immediate and thorough incorporation is also required for solid fractions of digestates to avoid NH_3 losses. Further, the considerable amount of easily available N has to be taken into account and application without corresponding crop N demand should be avoided because it bears the risk of NO_3^- leaching after nitrification of digestate N. The additional expenses caused by technical solid-liquid separation are not necessarily compensated by decreased NH_3 losses and an improved fertilizer value of the substrate. Immediate and complete incorporation of non separated digestate seems to be the most effective NH_3 mitigation measure. However, digestate separation might help to reduce costs for nutrient export from regions with intensive animal production and

nutrient surplus to regions specialized on cash crop production where no organic fertilizers such as animal manure are available.

The comparison of different digestates and animal slurries under field conditions further indicated a higher emission potential for digestates from maize (Chapter 2). High NH_3 losses from maize digestates were confirmed in the second field experiment where NH_3 emission reduced the fertilizer value of the digestate by nearly one third (Chapter 3). The results indicate that digestate-specific emission factors for NH_3 have to be developed. Using the same NH_3 emission factors as applied for animal manure may result in an underestimation of NH_3 losses from digestate application.

Regarding the total fertilizer-induced greenhouse gas balance of the treatments amended with digestates, NH_3 emission (including replacement of fertilizer-loss by mineral fertilizer) was the major contributor (Chapter 3). Hence, both field experiments confirmed that effective measures to reduce NH_3 emissions after digestate application are essential. Application with trail hoses which is recommended for growing crops still caused considerable NH_3 losses. Only immediate and complete digestate incorporation can minimize NH_3 emissions and preserve the potential fertilizer value of digestate. The results suggest that minimizing NH_3 emission from digestate application to growing crops is still a challenge which invites the development of new innovative techniques.

5.2 Importance and mitigation of N_2O emission following digestate application

In general, digestates can stimulate N_2O emissions from soil because they contain both large amounts of mineral N and a fraction of easily available organic C. In the laboratory incubation, nitrification of NH_4^+ from the maize digestate was fast and completed within less than three weeks (Chapter 4). The associated increase in soil NO_3^- can cause N_2O losses in periods with restricted aeration due to denitrification and also lead to NO_3^- leaching if the plant N uptake is not sufficient after digestate application to arable soil. The fraction of labile organic C in digestate resulted in strongly increased soil respiration rates after application and induced N_2O emission by denitrification (Chapter 4). The admixture of a nitrification inhibitor (NI) to digestates could help to retard the fast nitrification of digestate NH_4^+ and it may reduce N_2O losses by lowering soil NO_3^- concentration and by temporal separation of the periods with increased microbial O_2 consumption and NO_3^- formation from digestate N. In the field experiment (Chapter 3), the addition of the NI retarded nitrification and efficiently reduced N_2O emissions after digestate application for approx. 6 weeks both with and without substrate incorporation. In the rest of the year, no differences in N_2O emission among the treatments were measured. The soil of the field experiment (Chapter 3) had a low potential for direct N_2O emissions as only low emissions were measured apart from fertilizer-induced peaks. For this sandy soil under the prevalent climatic conditions, the NI was not able to reduce the total annual N_2O emission. However, the effect of NIs on N_2O emission are likely to vary under different soil and

climatic conditions and should therefore be further investigated with different soil types and weather conditions. In the context of climate protection the effect of NIs on the annual N_2O emission is relevant and short-term NIs effects on N_2O emission are not enough for their assessment.

Results from the second field experiment showed, that NH_3 emissions dominated N losses after digestate application (Chapter 3). Compared to those large N losses the amount of N emitted as N_2O was of minor relevance for the nutrient balance on the field. However, to avoid greenhouse gas emission from agriculture, mitigation of N_2O field emission is of great importance. The general low soil N_2O emission from the studied sandy soil could be mainly attributed to the low soil WFPS which was below the optimal level for denitrification. N_2O emissions are known to be largely influenced by the soil water content with higher emission at higher WFPS levels. This relation was confirmed by the low N_2O emissions from the treatment with mineral fertilizer application despite higher soil NO_3^- content during several weeks of the experiment. Our results on low annual N_2O emission may be typical for well aerated sandy soils but they should not be generalized because it is known that emissions can be much higher from other soil types and under other weather conditions.

The simulation of different application techniques in the laboratory study revealed only a small influence of different digestate incorporation techniques on total N_2O emissions (Chapter 4). Only surface application resulted in lower N_2O emission whereas both injection and incorporation showed no difference, possibly also due to low soil WFPS level. Nevertheless, surface application is not recommendable because of high NH_3 emission. The results of the laboratory incubation of ^{15}N labeled digestate revealed, that the application technique affected the dynamics of N_2O reduction and thus the emission of N_2 (Chapter 4). They suggest that the rapid formation of high NO_3^- contents in combination with restricted availability of digestate C limited the reduction of N_2O to N_2 and thus led to a higher proportion of N_2O emissions on total denitrification losses as compared with the incorporation of non digested maize biomass. However, these results have to be evaluated under field conditions where the quantification of N_2 emissions is still a challenge. Field studies are also important to determine the effect of different incorporation techniques under natural conditions and at an annual time scale. Since the application technique can influence the emissions of NH_3, N_2O and N_2 all these gases have to be taken into account to assess the environmental impact of different application techniques. N_2 emissions should be included because they represent a loss of plant available N and reduce the value of digestates as N fertilizer.

5.3 Carbon mineralization following the application of digestate

Carbon mineralization of fresh maize biomass and maize digestates was studied in a laboratory incubation with different application techniques (Chapter 4). Digestate C was more resistant to decomposition and the application type had no influence on total C mineralization. However, the

digestate contained an organic fraction which was rapidly mineralized within two weeks after application. Further differentiation of C pools within the digestate revealed considerable differences between the less and the more processed C pool (i.e. substrate with shorter and longer residence time in the digester). The less processed C pool (derived from labeled maize) showed highest mineralization rates and was preferentially decomposed after soil application. This indicates that the ability of a digestate to maintain or even increase soil C levels after application depends on the degree of decomposition in the digester. This, however, differs among different substrates and should be investigated for different input materials.

The value of digestate for replenishment or sequestration of soil organic matter consequently increases with prolonged processing in the digester. The amount of organic C which can be applied to soils with digestates is generally restricted by their high nutrient contents because nutrient surplus has to be avoided very strictly. To evaluate the long-term effects of different digestates on soil organic matter turnover and C sequestration, studies with repeated digestate application over several years are required. These studies may also help to assess the function of organic digestate N as a slow source of mineral N and to evaluate the long-term effect of soil organic matter replenishment via digestate on soil N dynamics.

The results show that anoxic organic C stabilization processes in the digester result in a fraction which can be rapidly mineralized under oxic conditions by soil microbes. This labile fraction of digestates plays an important role for N_2O emission as it strongly increases O_2 consumption in soil and thus triggers denitrification of both soil NO_3^- and NO_3^- produced from digestate N.

To conclude, digestates from energy crops are valuable N fertilizers if NH_3 losses are efficiently avoided. In particular in regions where fertilization is mainly based on synthetic N fertilizers, digestates can substitute these synthetic fertilizers and contribute to a sustainable management of nutrients and of soil organic matter. Measures to minimize NH_3 emission from digestates should become obligatory to avoid negative effects of biogas production on environment and climate. However, NH_3 mitigation cannot solve the general problem of nutrient surpluses (in particular N and P) in some regions with intensive animal production. This problem has to be addressed by transparent and serious field based nutrient budgets. It is essential to include all effects of NH_3 mitigation measures in the farm and field N budgets in order to account for the higher fertilizer value. This will contribute to more realistic N budgets even if the calculatory N surplus is probably getting worse in some regions.

This thesis addresses the emission of NH_3 and N_2O after application of digestate. However, this represents only a part of the whole biogas production chain where potential leakages for harmful emissions can occur at several steps. Well known examples are CH_4 emissions from the digester and CH_4 and NH_3 emissions from storage of digestate. Thus, minimizing emissions from biogas production requires a system approach including the whole production chain. An optimized digestate management is one of the keys for a more sustainable biogas production.

6 References

Abubaker J, Odlare M, Pell M (2013) Nitrous Oxide Production from Soils Amended with Biogas Residues and Cattle Slurry. Journal of Environmental Quality 42(4): 1046-1058

Akiyama H, Yan X, Yagi K (2010) Evaluation of effectiveness of enhanced-efficiency fertilizers as mitigation options for N_2O and NO emissions from agricultural soils: meta-analysis. Global Change Biology 16(6): 1837-1846

Alburquerque JA, de la Fuente C, Bernal MP (2012) Chemical properties of anaerobic digestates affecting C and N dynamics in amended soils. Agriculture, Ecosystems & Environment 160(0): 15-22

Amon B, Kryvoruchko V, Amon T, Zechmeister-Boltenstern S (2006) Methane, nitrous oxide and ammonia emissions during storage and after application of dairy cattle slurry and influence of slurry treatment. Agriculture Ecosystems & Environment 112(2-3): 153-162

Amon T, Amon B, Kryvoruchko V, Zollitsch W, Mayer K, Gruber L (2007) Biogas production from maize and dairy cattle manure - Influence of biomass composition on the methane yield. Agriculture Ecosystems & Environment 118(1-4): 173-182

Appels L, Lauwers J, Degreve J, Helsen L, Lievens B, Willems K, Van Impe J, Dewil R (2011) Anaerobic digestion in global bio-energy production: Potential and research challenges. Renewable & Sustainable Energy Reviews 15(9): 4295-4301

Arthurson V (2009) Closing the Global Energy and Nutrient Cycles through Application of Biogas Residue to Agricultural Land - Potential Benefits and Drawbacks. Energies 2(2): 226-242

Asing J, Saggar S, Singh J, Bolan NS (2008) Assessment of nitrogen losses from urea and an organic manure with and without nitrification inhibitor, dicyandiamide, applied to lettuce under glasshouse conditions. Australian Journal of Soil Research 46(6-7): 535-541

Asman WAH, Sutton MA, Schjorring JK (1998) Ammonia: emission, atmospheric transport and deposition. New Phytol. 139(1): 27-48

Asmus A, Linke B, Dunkel H (1988) Eigenschaften und Düngerwirkung von ausgefaulter Gülle aus der Biogasgewinnung. Archiv für Acker- und Pflanzenbau und Bodenkunde, Berlin 32: 527-532

Baggs EM, Philippot L (2010) Microbial Terrestrial Pathway to Nitrous Oxide. In: Smith K (ed) Nitrous Oxide and Climate Change. Earthscan, London, UK.

Balsari P, Dinuccio E, Santoro E, Gioelli F (2008) Ammonia emissions from rough cattle slurry and from derived solid and liquid fractions applied to alfalfa pasture. Aust. J. Exp. Agric. 48(1-2): 198-201

Barton L, McLay CDA, Schipper LA, Smith CT (1999) Annual denitrification rates in agricultural and forest soils: a review. Australian Journal of Soil Research 37(6): 1073-1093

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate - a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society Series B-Methodological 57(1): 289-300

Benjamini Y, Yekutieli D (2001) The control of the false discovery rate in multiple testing under dependency. Annals of Statistics 29(4): 1165-1188

Bernal MP, Kirchmann H (1992) Carbon and nitrogen mineralization and ammonia volatilization from fresh, aerobically and anaerobically treated pig manure during incubation with soil. Biol. Fertil. Soils 13(3): 135-141

Bertora C, Alluvione F, Zavattaro L, van Groenigen JW, Velthof G, Grignani C (2008) Pig slurry treatment modifies slurry composition, N_2O, and CO_2 emissions after soil incorporation. Soil Biol. Biochem. 40(8): 1999-2006

Beusen AHW, Bouwman AF, Heuberger PSC, Van Drecht G, Van Der Hoek KW (2008) Bottom-up uncertainty estimates of global ammonia emissions from global agricultural production systems. Atmos. Environ. 42(24): 6067-6077

Blackmer AM, Bremner JM (1978) Inhibitory Effect of Nitrate on Reduction of N_2O to N_2 by Soil-Microorganisms. Soil Biol. Biochem. 10(3): 187-191

Blagodatskaya E, Kuzyakov Y (2008) Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. Biol. Fertil. Soils 45(2): 115-131

BMU (2010) Nationaler Biomasseaktionsplan für Deutschland. Beitrag der Biomasse für eine nachhaltige Energieversorgung. In. Bundesministerium für Umwelt, Naturschutz und Reaktorsicherheit (BMU), Bundesministerium für Ernährung und Landwirtschaft (BMEL), Berlin.

BMU (2013) Renewable Energy Sources in Figures - National and International Development. In. Federal Ministry for the Environment, Nature Conservation and Nuclear Safety (BMU), Berlin, Germany.

Bouwman AF, Boumans LJM, Batjes NH (2002a) Emissions of N_2O and NO from fertilized fields: Summary of available measurement data. Global Biogeochem. Cycles 16(4):

Bouwman AF, Boumans LJM, Batjes NH (2002b) Estimation of global NH_3 volatilization loss from synthetic fertilizers and animal manure applied to arable lands and grasslands. Global Biogeochem. Cycles 16(2):

Bouwman L, Stehfest E, van Kessel C (2010) Nitrous oxide emissions from the nitrogen cycle in arable agriculture: estimation and mitigation. In: Smith K (ed) Nitrous oxide and climate change. Earthscan, London, UK.

Braschkat J, Mannheim T, Marschner H (1997) Estimation of ammonia losses after application of liquid cattle manure on grassland. Z. Pflanzen. Bodenk. 160(2): 117-123

Bremner JM (1997) Sources of nitrous oxide in soils. Nutrient Cycling in Agroecosystems 49(1-3): 7-16

Bremner JM, Blackmer AM (1978) Nitrous Oxide - Emission from Soils During Nitrification of Fertilizer Nitrogen. Science 199(4326): 295-296

Brentrup F, Pallière C (2008) GHG emissions and energy efficiency in European nitrogen fertiliser production and use. International Fertilizer Society,

Cayuela ML, Oenema O, Kuikman PJ, Bakker RR, van Groenigen JW (2010) Bioenergy by-products as soil amendments? Implications for carbon sequestration and greenhouse gas emissions. Global Change Biology Bioenergy 2(4): 201-213

Chantigny MH, Angers DA, Belanger G, Rochette P, Eriksen-Hamel N, Bittman S, Buckley K, Masse D, Gasser M-O (2008) Yield and nutrient export of grain corn fertilized with raw and treated liquid swine manure. Agronomy Journal 100(5): 1303-1309

Chantigny MH, Angers DA, Rochette P, Belanger G, Masse D, Cote D (2007) Gaseous nitrogen emissions and forage nitrogen uptake on soils fertilized with raw and treated swine manure. Journal of Environmental Quality 36(6): 1864-1872

Chantigny MH, MacDonald JD, Beaupre C, Rochette P, Angers DA, Masse D, Parent LE (2009) Ammonia volatilization following surface application of raw and treated liquid swine manure. Nutrient Cycling in Agroecosystems 85(3): 275-286

Chen R, Blagodatskaya E, Senbayram M, Blagodatsky S, Myachina O, Dittert K, Kuzyakov Y (2012) Decomposition of biogas residues in soil and their effects on microbial growth kinetics and enzyme activities. Biomass & Bioenergy 45: 221-229

Christensen ML, Hjorth M, Keiding K (2009) Characterization of pig slurry with reference to flocculation and separation. Water Research 43(3): 773-783

Ciais P, Sabine C, Bala G, Bopp L, Brovkin V, Canadell J, Chhabra A, DeFries R, Galloway J, Heimann M, Jones C, Quéré CL, Myneni RB, Piao S, Thornton P (2013) Carbon and Other Biogeochemical Cycles. In: Stocker TF, D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (ed) Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Clemens J, Huschka A (2001) The effect of biological oxygen demand of cattle slurry and soil moisture on nitrous oxide emissions. Nutrient Cycling in Agroecosystems 59(2): 193-198

Clemens J, Trimborn M, Weiland P, Amon B (2006) Mitigation of greenhouse gas emissions by anaerobic digestion of cattle slurry. Agriculture Ecosystems & Environment 112(2-3): 171-177

Comfort SD, Kelling KA, Keeney DR, Converse JC (1990) Nitrous-oxide production from injected liquid dairy manure. Soil Sci. Soc. Am. J. 54(2): 421-427

Coppens F, Garnier P, De Gryze S, Merckx R, Recous S (2006) Soil moisture, carbon and nitrogen dynamics following incorporation and surface application of labelled crop residues in soil columns. Eur. J. Soil Sci. 57(6): 894-905

Cornforth IS, Chesney HAD (1971) Nitrification inhibitors and ammonia volatilization. Plant and Soil 34: 497-501

Correll DL (1998) The role of phosphorus in the eutrophication of receiving waters: A review. Journal of Environmental Quality 27(2): 261-266

Davidson EA (1991) Fluxes of nitrous oxide and nitric oxide from terrestrial ecosystems. In: Rogers JE & Whitman WB (eds) Microbial production and consumption of greenhouse gases ; Methane, nitrogen oxides, and halomethanes. Am. Soc. Microbiol., Washington, D.C. p 219-235

de Boer HC (2008) Co-digestion of animal slurry can increase short-term nitrogen recovery by crops. Journal of Environmental Quality 37(5): 1968-1973

de la Fuente C, Alburquerque JA, Clemente R, Bernal MP (2013) Soil C and N mineralisation and agricultural value of the products of an anaerobic digestion system. Biol. Fertil. Soils 49(3): 313-322

deKlein CAM, vanLogtestijn RSP, vandeMeer HG, Geurink JH (1996) Nitrogen losses due to denitrification from cattle slurry injected into grassland soil with and without a nitrification inhibitor. Plant and Soil 183(2): 161-170

Dendooven L, Bonhomme E, Merckx R, Vlassak K (1998) Injection of pig slurry and its effects on dynamics of nitrogen and carbon in a loamy soil under laboratory conditions. Biol. Fertil. Soils 27(1): 5-8

Dendooven L, Splatt P, Anderson JM (1996) Denitrification in permanent pasture soil as affected by different forms of C substrate. Soil Biol. Biochem. 28(2): 141-149

Denman KL, Brasseur G., Chidthaisong A., Ciais P., Cox P.M, Dickinson R.E., Hauglustaine D., Heinze C., Holland E., Jacob D., Lohmann U., Ramachandran S., da Silva Dias P.L., Wofsy S.C., X. Z (2007) Couplings Between Changes in the Climate System and Biogeochemistry. In:

Solomon S. QD, Manning M., Chen Z., Marquis M., Averyt K.B., Tignor M., Miller H.L. (ed) Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climat Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Dinuccio E, Gioelli F, Balsari P, Dorno N (2012) Ammonia losses from the storage and application of raw and chemo-mechanically separated slurry. Agriculture Ecosystems & Environment 153: 16-23

Dittert K, Bol R, King R, Chadwick D, Hatch D (2001) Use of a novel nitrification inhibitor to reduce nitrous oxide emission from N-15-labelled dairy slurry injected into soil. Rapid Commun. Mass Spectrom. 15(15): 1291-1296

Dobbie KE, McTaggart IP, Smith KA (1999) Nitrous oxide emissions from intensive agricultural systems: Variations between crops and seasons, key driving variables, and mean emission factors. J. Geophys. Res. Atmos. 104(D21): 26891-26899

Dobbie KE, Smith KA (2003a) Impact of different forms of N fertilizer on N_2O emissions from intensive grassland. Nutrient Cycling in Agroecosystems 67(1): 37-46

Dobbie KE, Smith KA (2003b) Nitrous oxide emission factors for agricultural soils in Great Britain: the impact of soil water-filled pore space and other controlling variables. Global Change Biology 9(2): 204-218

Dorno N, Feilberg A, Balsari P, Nyord T (2013) Nitrous oxide losses from untreated and digested slurry as influenced by soil moisture and application method. Biosystems Engineering 115(4): 423-433

Dörsch P, Palojarvi A, Mommertz S (2004) Overwinter greenhouse gas fluxes in two contrasting agricultural habitats. Nutrient Cycling in Agroecosystems 70(2): 117-133

Dosch P, Gutser R (1996) Reducing N losses (NH_3, N_2O, N_2) and immobilization from slurry through optimized application techniques. Fertilizer Research 43(1-3): 165-171

Ebertseder T, Groß J (2008) Ausweitung des Energiepflanzenanbaus aus Sicht des Pflanzenbaus und des Nährstoffmanagements. In: Klimawandel und Bioenergie - Pflanzenproduktion im Spannungsfeld zwischen politischen Vorgaben und Ökonomischen Rahmenbedingungen. Bundesarbeitskreis Düngung (BAD), Frankfurt.

EEA (2009) EMEP/EEA air pollutant emission inventory guidebook 2009; Technical guidance to prepare national emission inventories. Copenhagen, Denmark

EEA (2010) Revealing the costs of air pollution from industrial facilities in Europe, EEA Technical report No 15/2011. European Environmental Agency, Copenhagen, Denmark

Eichner MJ (1990) Nitrous Oxide Emissions from Fertilized Soils - Summary of Available Data. Journal of Environmental Quality 19(2): 272-280

EurObserv'er (2011) The State of Renewable Energies in Europe. 12th EurObser'ER Report. In., Paris. p 55-61

EurObserv'er (2013) The State of Renewable Energies in Europe, Edition 2013. In., Paris. p 44-49

Fangueiro D, Chadwick D, Dixon L, Grilo J, Walter N, Bol R (2010) Short term N_2O, CH_4 and CO_2 production from soil sampled at different depths and amended with a fine sized slurry fraction. Chemosphere 81(1): 100-108

Ferm M (1998) Atmospheric ammonia and ammonium transport in Europe and critical loads: a review. Nutrient Cycling in Agroecosystems 51(1): 5-17

Ferm M, Kasimir-Klemedtsson A, Weslien P, Klemedtsson L (1999) Emission of NH_3 and N_2O after spreading of pig slurry by broadcasting or band spreading. Soil Use Manage. 15(1): 27-33

Field JA, Reneau RB, Kroontje W, Caldwell JS, Jeyanayagam S, Collins ER (1984) Fertilizer recovery from anaerobic digesters for poultry, dairy, and swine manure. Paper, American Society of Agricultural Engineers (84-2109): pp-28 pp

Flessa H, Beese F (1995) Effects of sugar-beet residues on soil redox potential and nitrous-oxide emission. Soil Sci. Soc. Am. J. 59(4): 1044-1051

Flessa H, Beese F (2000) Laboratory estimates of trace gas emissions following surface application and injection of cattle slurry. Journal of Environmental Quality 29(1): 262-268

Flessa H, Dorsch P, Beese F (1995) Seasonal-variation of N_2O and CH_4 fluxes in differently managed arable soils in southern Germany. J. Geophys. Res. Atmos. 100(D11): 23115-23124

Flessa H, Ruser R, Schilling R, Loftfield N, Munch JC, Kaiser EA, Beese F (2002) N_2O and CH_4 fluxes in potato fields: automated measurement, management effects and temporal variation. Geoderma 105(3-4): 307-325

FNR (2013) Basisdaten Bioenergie Deutschland August 2013. Fachagentur Nachwachsende Rohstoffe e.V., Gülzow, Germany

Folorunso OA, Rolston DE (1984) Spatial variability of field-measured denitrification gas fluxes. Soil Sci. Soc. Am. J. 48(6): 1214-1219

Forster P, Ramaswamy V, Artaxo P, Berntsen T, Betts R, Fahey DW, Haywood J, Lean J, Lowe DC, Myhre G, Nganga J, Prinn R, Raga G, Schulz M, Dorland RV (2007) Changes in Atmospheric Constituents and in Radiative Forcing. In: Solomon S, D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M.Tignor & Miller HL (eds) Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Fouda S, von Tucher S, Lichti F, Schmidhalter U (2013) Nitrogen availability of various biogas residues applied to ryegrass. Journal of Plant Nutrition and Soil Science 176(4): 572-584

Freney JR, Simpson JR, Denmead OT (1983) Volatilization of Ammonia. In: Freney JR & Simpson JR (eds) Gaseous Loss of Nitrogen from Plant-Soil Systems. Developments in Plant and Soil Sciences, vol Volume 9. Kluwer Academic Publishers Group, Dordrecht, The Netherlands.

Frost JP, Stevens RJ, Laughlin RJ (1990) Effect of separation and acidification of cattle slurry on ammonia volatilization and on the efficiency of slurry nitrogen for herbage production. Journal of Agricultural Science 115: 49-56

Gemmeke B, Rieger C, Weiland P (2009) Biogas Messprogramm II, 61 Biogasanlagen im Vergleich. In. Fachagentur Nachwachsende Rohstoffe (FNR), Gülzow, Germany.

Gericke D, Bornemann L, Kage H, Pacholski A (2012) Modelling Ammonia Losses After Field Application of Biogas Slurry in Energy Crop Rotations. Water Air and Soil Pollution 223(1): 29-47

Gericke D, Pacholski A, Kage H (2011) Measurement of ammonia emissions in multi-plot field experiments. Biosystems Engineering 108(2): 164-173

Gordon R, Jamieson R, Rodd V, Patterson G, Harz T (2001) Effects of surface manure application timing on ammonia volatilization. Can. J. Soil Sci. 81(3): 525-533

Grigatti M, Di Girolamo G, Chincarini R, Ciavatta C, Barbanti L (2011) Potential nitrogen mineralization, plant utilization efficiency and soil CO_2 emissions following the addition of anaerobic digested slurries. Biomass & Bioenergy 35(11): 4619-4629

Gutser R, Ebertseder T, Weber A, Schraml M, Schmidhalter U (2005) Short-term and residual availability of nitrogen after long-term application of organic fertilizers on arable land. J. Plant Nutr. Soil Sci. 168(4): 439-446

Hansen MN, Henriksen K, Sommer SG (2006) Observations of production and emission of greenhouse gases and ammonia during storage of solids separated from pig slurry: Effects of covering. Atmos. Environ. 40(22): 4172-4181

Hantschel RE, Flessa H, Beese F (1994) An automated microcosm system for studying soil ecological processes. Soil Sci. Soc. Am. J. 58(2): 401-404

Haynes RJ, Naidu R (1998) Influence of lime, fertilizer and manure applications on soil organic matter content and soil physical conditions: a review. Nutrient Cycling in Agroecosystems 51(2): 123-137

Henriksen TM, Breland TA (2002) Carbon mineralization, fungal and bacterial growth, and enzyme activities as affected by contact between crop residues and soil. Biol. Fertil. Soils 35(1): 41-48

Herrmann A (2013) Biogas Production from Maize: Current State, Challenges and Prospects. 2. Agronomic and Environmental Aspects. Bioenergy Research 6(1): 372-387

Hjorth M, Christensen KV, Christensen ML, Sommer SG (2010) Solid-liquid separation of animal slurry in theory and practice. A review. Agronomy for Sustainable Development 30(1): 153-180

Holm-Nielsen JB, Al Seadi T, Oleskowicz-Popiel P (2009) The future of anaerobic digestion and biogas utilization. Bioresour. Technol. 100(22): 5478-5484

Hothorn T, Bretz F, Westfall P (2008) Simultaneous Inference in General Parametric Models. Biometrical Journal 50(3):

Huijsmans JFM, Hol JMG, Vermeulen GD (2003) Effect of application method, manure characteristics, weather and field conditions on ammonia volatilization from manure applied to arable land. Atmos. Environ. 37(26): 3669-3680

Hutchings N, Amon B, Dämmgen U, Webb J (2013) EMEP/EEA air pollutant emission inventory guidebook 2013, Part B: Technical chapters, Agriculture. In: EMEP/EEA air pollutant emission inventory guidebook 2013. European Environment Agency, Luxembourg.

Hutchinson GL, Mosier AR (1981) Improved soil cover method for field measurement of nitrous-oxide fluxes. Soil Sci. Soc. Am. J. 45(2): 311-316

IPCC (2006) 2006 IPCC Guidelines for National Greenhouse Gas Inventories, Prepared by the National Greenhouse Gas Inventories Programme. Institute for Global Environmental Studies (IGES), Japan

Jaeger N, Duffner A, Ludwig B, Flessa H (2013) Effect of fertilization history on short-term emission of CO_2 and N_2O after the application of different N fertilizers - a laboratory study. Archives of Agronomy and Soil Science 59(2): 161-171

Jaeger N, Stange CF, Ludwig B, Flessa H (2011) Emission rates of N_2O and CO_2 from soils with different organic matter content from three long-term fertilization experiments-a laboratory study. Biol. Fertil. Soils 47(5): 483-494

Jarecki MK, Parkin TB, Chan ASK, Hatfield JL, Jones R (2008) Greenhouse gas emissions from two soils receiving nitrogen fertilizer and swine manure slurry. Journal of Environmental Quality 37(4): 1432-1438

Johansen A, Carter MS, Jensen ES, Hauggard-Nielsen H, Ambus P (2013) Effects of digestate from anaerobically digested cattle slurry and plant materials on soil microbial community and emission of CO_2 and N_2O. Applied Soil Ecology 63: 36-44

Jungkunst HF, Freibauer A, Neufeldt H, Bareth G (2006) Nitrous oxide emissions from agricultural land use in Germany - a synthesis of available annual field data. J. Plant Nutr. Soil Sci. 169(3): 341-351

Kai P, Pedersen P, Jensen JE, Hansen MN, Sommer SG (2008) A whole-farm assessment of the efficacy of slurry acidification in reducing ammonia emissions. European Journal of Agronomy 28(2): 148-154

Kaiser EA, Ruser R (2000) Nitrous oxide emissions from arable soils in Germany - An evaluation of six long-term field experiments. Journal of Plant Nutrition and Soil Science 163(3): 249-259

Kim D-G, Saggar S, Roudier P (2012) The effect of nitrification inhibitors on soil ammonia emissions in nitrogen managed soils: a meta-analysis. Nutrient Cycling in Agroecosystems 93(1): 51-64

Kirchmann H (1991) Carbon and nitrogen mineralization of fresh, anerobic and anaerobic animal manures during incubation with soil. Swedish Journal of Agricultural Research 21(4): 165-173

Kirchmann H, Lundvall A (1993) Relationship between N-immobilization and volatile fatty-acids in soil after application of pig and cattle slurry. Biol. Fertil. Soils 15(3): 161-164

Kirchmann H, Witter E (1992) Composition of fresh, aerobic and anaerobic farm animal dungs. Bioresour. Technol. 40(2): 137-142

Knowles R (1982) Denitrification in soils. Advances in agricultural microbiology: 243-266

Kocar G (2008) Anaerobic digesters: From waste to energy crops as an alternative energy source. Energy Sources Part a-Recovery Utilization and Environmental Effects 30(7): 660-669

Koester JR, Cardenas L, Senbayram M, Bol R, Well R, Butler M, Muehling KH, Dittert K (2011) Rapid shift from denitrification to nitrification in soil after biogas residue application as indicated by nitrous oxide isotopomers. Soil Biol. Biochem. 43(8): 1671-1677

Körschens M (2004) Humusbilanzierung: Methode zur Beurteilung und Bemessung der Humusversorgung von Ackerland. Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten (VDLUFA), Bonn, Germany

Kuzyakov Y, Friedel JK, Stahr K (2000) Review of mechanisms and quantification of priming effects. Soil Biol. Biochem. 32(11-12): 1485-1498

Lal R (2001) World cropland soils as a source or sink for atmospheric carbon. In: Sparks DL (ed). Advances in Agronomy. p 145-191

Levi-Minzi R, Riffaldi R, Saviozzi A (1990) Carbon mineralization in soil amended with different organic materials. Agriculture, Ecosystems & Environment 31(4): 325-335

Lewicka-Szczebak D, Well R, Giesemann A, Rohe L, Wolf U (2013) An enhanced technique for automated determination of N-15 signatures of N_2, (N_2+N_2O) and N_2O in gas samples. Rapid Commun. Mass Spectrom. 27(13): 1548-1558

Loftfield N, Flessa H, Augustin J, Beese F (1997) Automated gas chromatographic system for rapid analysis of the atmospheric trace gases methane, carbon dioxide, and nitrous oxide. Journal of Environmental Quality 26(2): 560-564

Lorenz K, Lal R, Preston CM, Nierop KGJ (2007) Strengthening the soil organic carbon pool by increasing contributions from recalcitrant aliphatic bio(macro)molecules. Geoderma 142(1-2): 1-10

Lovanh N, Warren J, Sistani K (2010) Determination of ammonia and greenhouse gas emissions from land application of swine slurry: A comparison of three application methods. Bioresour. Technol. 101(6): 1662-1667

Macadam XMB, del Prado A, Merino P, Estavillo JM, Pinto M, Gonzalez-Murua C (2003) Dicyandiamide and 3,4-dimethyl pyrazole phosphate decrease N_2O emissions from grassland but dicyandiamide produces deleterious effects in clover. Journal of Plant Physiology 160(12): 1517-1523

Malgeryd J (1998) Technical measures to reduce ammonia losses after spreading of animal manure. Nutrient Cycling in Agroecosystems 51(1): 51-57

Marcato C-E, Mohtar R, Revel J-C, Pouech P, Hafidi M, Guiresse M (2009) Impact of anaerobic digestion on organic matter quality in pig slurry. International Biodeterioration & Biodegradation 63(3): 260-266

Markfoged R, Nielsen LP, Nyord T, Ottosen LDM, Revsbech NP (2011) Transient N_2O accumulation and emission caused by O_2 depletion in soil after liquid manure injection. Eur. J. Soil Sci. 62(4): 541-550

McKenney DJ, Wang SW, Drury CF, Findlay WI (1993) Denitrification and mineralization in soil amended with legume, grass and corn residues. Soil Sci. Soc. Am. J. 57(4): 1013-1020

McTaggart IP, Clayton H, Parker J, Swan L, Smith KA (1997) Nitrous oxide emissions from grassland and spring barley, following N fertiliser application with and without nitrification inhibitors. Biol. Fertil. Soils 25(3): 261-268

Meade G, Pierce K, O'Doherty JV, Mueller C, Lanigan G, Mc Cabe T (2011) Ammonia and nitrous oxide emissions following land application of high and low nitrogen pig manures to winter wheat at three growth stages. Agriculture Ecosystems & Environment 140(1-2): 208-217

Meijide A, Diez JA, Sanchez-Martin L, Lopez-Fernandez S, Vallejo A (2007) Nitrogen oxide emissions from an irrigated maize crop amended with treated pig slurries and composts in a Mediterranean climate. Agriculture Ecosystems & Environment 121(4): 383-394

Menardo S, Gioelli F, Balsari P (2011) The methane yield of digestate: Effect of organic loading rate, hydraulic retention time, and plant feeding. Bioresour. Technol. 102(3): 2348-2351

Menendez S, Merino P, Pinto M, Gonzalez-Murua C, Estavillo JM (2006) 3,4-dimethylpyrazol phosphate effect on nitrous oxide, nitric oxide, ammonia, and carbon dioxide emissions from grasslands. Journal of Environmental Quality 35(4): 973-981

Merino P, Estavillo JM, Graciolli LA, Pinto M, Lacuesta M, Munoz-Rueda A, Gonzalez-Murua C (2002) Mitigation of N_2O emissions from grassland by nitrification inhibitor and Actilith F2 applied with fertilizer and cattle slurry. Soil Use Manage. 18(2): 135-141

Merino P, Menendez S, Pinto M, Gonzalez-Murua C, Estavillo JM (2005) 3,4-dimethylpyrazole phosphate reduces nitrous oxide emissions from grassland after slurry application. Soil Use Manage. 21(1): 53-57

Meyer-Aurich A, Schattauer A, Hellebrand HJ, Klauss H, Ploechl M, Berg W (2012) Impact of uncertainties on greenhouse gas mitigation potential of biogas production from agricultural resources. Renewable Energy 37(1): 277-284

Meyer A, Bergmann J, Butterbach-Bahl K, Brueggemann N (2010) A new 15N tracer method to determine N turnover and denitrification of Pseudomonas stutzeri. Isotopes in Environmental and Health Studies 46(4): 409-421

Misselbrook TH, Brookman SKE, Smith KA, Cumby T, Williams AG, McCrory DF (2005) Crusting of stored dairy slurry to abate ammonia emissions: Pilot-scale studies. Journal of Environmental Quality 34(2): 411-419

Misselbrook TH, Smith KA, Johnson RA, Pain BF (2002) Slurry application techniques to reduce ammonia emissions: Results of some UK field-scale experiments. Biosystems Engineering 81(3): 313-321

Moitzi G, Amon B, Amon T, Kryvoruchko V, Wagner-Alt C, Hackl E, Zechmeister-Boltenstern S, Boxberger J (2007) Emissions of NH_3, CH_4 and N_2O during storage and after application of untreated and anaerobically digested slurry. Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Agriculture 63/64: 368-373

Moller HB, Lund I, Sommer SG (2000) Solid-liquid separation of livestock slurry: efficiency and cost. Bioresour. Technol. 74(3): 223-229

Moller HB, Sommer SG, Ahring BK (2002) Separation efficiency and particle size distribution in relation to manure type and storage conditions. Bioresour. Technol. 85(2): 189-196

Möller K, Müller T (2012) Effects of anaerobic digestion on digestate nutrient availability and crop growth: A review. Eng. Life Sci. 12(3): 242-257

Möller K, Schulz R, Müller T (2011) Effects of setup of centralized biogas plants on crop acreage and balances of nutrients and soil humus. Nutrient Cycling in Agroecosystems 89(2): 303-312

Möller K, Stinner W (2009) Effects of different manuring systems with and without biogas digestion on soil mineral nitrogen content and on gaseous nitrogen losses (ammonia, nitrous oxides). European Journal of Agronomy 30(1): 1-16

Möller K, Stinner W, Deuker A, Leithold G (2008) Effects of different manuring systems with and without biogas digestion on nitrogen cycle and crop yield in mixed organic dairy farming systems. Nutrient Cycling in Agroecosystems 82(3): 209-232

Morvan T, Nicolardot B, Pean L (2006) Biochemical composition and kinetics of C and N mineralization of animal wastes: a typological approach. Biol. Fertil. Soils 42(6): 513-522

Mosier AR, Duxbury JM, Freney JR, Heinemeyer O, Minami K (1996) Nitrous oxide emissions from agricultural fields: Assessment, measurement and mitigation. Plant and Soil 181(1): 95-108

Murphy J, Braun R, Weiland P, Wellinger A (2011) Biogas from Crop Digestion. IEA Bioenergy Task 37 - Energy from Biomass:

Ni K, Pacholski A, Gericke D, Kage H (2012) Analysis of ammonia losses after field application of biogas slurries by an empirical model. Journal of Plant Nutrition and Soil Science 175(2): 253-264

Nielsen TH, Nielsen LP, Revsbech NP (1996) Nitrification and coupled nitrification-denitrification associated with a soil-manure interface. Soil Sci. Soc. Am. J. 60(6): 1829-1840

Nyord T, Hansen MN, Birkmose TS (2012) Ammonia volatilisation and crop yield following land application of solid-liquid separated, anaerobically digested, and soil injected animal slurry to winter wheat. Agriculture Ecosystems & Environment 160: 75-81

Odlare M, Pell M, Svensson K (2008) Changes in soil chemical and microbiological properties during 4 years of application of various organic residues. Waste Management 28(7): 1246-1253

Oquist MG, Nilsson M, Sorensson F, Kasimir-Klemedtsson A, Persson T, Weslien P, Klemedtsson L (2004) Nitrous oxide production in a forest soil at low temperatures - processes and environmental controls. Fems Microbiology Ecology 49(3): 371-378

Pacholski A, Cai G-X, Fan X-H, Ding H, Chen D, Nieder R, Roelcke M (2008) Comparison of different methods for the measurement of ammonia voltatilization after urea application in Henan Province, China. Journal of Plant Nutrition and Soil Science 171(3): 361-369

Pacholski A, Cai GX, Nieder R, Richter J, Fan XH, Zhu ZL, Roelcke M (2006) Calibration of a simple method for determining ammonia volatilization in the field - comparative measurements in Henan Province, China. Nutrient Cycling in Agroecosystems 74(3): 259-273

Pacholski A, Gericke D, Ni K, Kage H (2010) Ammoniakemissionen nach Ausbringung von Gärresten im Vergleich zu Güllen. Kuratorium für Technik und Bauwesen in der Landwirtschaft e.V. (KTBL), Darmstadt,

Pain BF, Misselbrook TH, Clarkson CR, Rees YJ (1990a) Odor and ammonia emissions following the spreading of anaerobically-digested pig slurry on grassland. Biological Wastes 34(3): 259-267

Pain BF, Phillips VR, Clarkson CR, Klarenbeek JV (1989) Loss of nitrogen through ammonia volatilization during and following the application of pig or cattle slurry to grassland. Journal of the Science of Food and Agriculture 47(1): 1-12

Pain BF, Phillips VR, Clarkson CR, Misselbrook TH, Rees YJ, Farrent JW (1990b) Odor and ammonia emissions following the spreading of aerobically-treated pig slurry on grassland. Biological Wastes 34(2): 149-160

Pain BF, Thompson RB, Rees YJ, Skinner JH (1990c) Reducing gaseous losses from cattle slurry applied to grassland by the use of additives. Journal of the Science of Food and Agriculture 50(2): 141-153

Parkin TB, Hatfield JL (2010) Influence of nitrapyrin on N2O losses from soil receiving fall-applied anhydrous ammonia. Agriculture Ecosystems & Environment 136(1-2): 81-86

Paul JW, Beauchamp EG (1989) Relationship between volatile fatty-acids, total ammonia and pH in manure slurries. Biological Wastes 29(4): 313-318

Pelster DE, Chantigny MH, Rochette P, Angers DA, Rieux C, Vanasse A (2012) Nitrous Oxide Emissions Respond Differently to Mineral and Organic Nitrogen Sources in Contrasting Soil Types. Journal of Environmental Quality 41(2): 427-435

Perala P, Kapuinen P, Esala M, Tyynela S, Regina K (2006) Influence of slurry and mineral fertiliser application techniques on N_2O and CH_4 fluxes from a barley field in southern Finland. Agriculture Ecosystems & Environment 117(1): 71-78

Petersen SO (1992) Nitrification and denitrification after direct injection of liquid cattle manure. Acta Agriculturae Scandinavica Section B-Soil and Plant Science 42(2): 94-99

Petersen SO (1999) Nitrous oxide emissions from manure and inorganic fertilizers applied to spring barley. Journal of Environmental Quality 28(5): 1610-1618

Petersen SO, Nielsen TH, Frostegard A, Olesen T (1996) O_2 uptake, C metabolism and denitrification associated with manure hot-spots. Soil Biol. Biochem. 28(3): 341-349

Petersen SO, Nissen HH, Lund I, Ambus P (2003) Redistribution of slurry components as influenced by injection method, soil, and slurry properties. Journal of Environmental Quality 32(6): 2399-2409

Pfab H, Palmer I, Buegger F, Fiedler S, Mueller T, Ruser R (2012) Influence of a nitrification inhibitor and of placed N-fertilization on N_2O fluxes from a vegetable cropped loamy soil. Agriculture Ecosystems & Environment 150: 91-101

Pinheiro J, Bates D, DebRoy S, Sarkar D, Team TRDC (2012) nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-104,

Prasad R, Power JF (1995) Nitrification inhibitors for agriculture, health, and the environment. Advances in Agronomy, Vol 54 54: 233-281

Provenzano MR, Malerba AD, Pezzolla D, Gigliotti G (2014) Chemical and spectroscopic characterization of organic matter during the anaerobic digestion and successive composting of pig slurry. Waste Management 34(3): 653-660

Quakernack R, Pacholski A, Techow A, Herrmann A, Taube F, Kage H (2012) Ammonia volatilization and yield response of energy crops after fertilization with biogas residues in a coastal marsh of Northern Germany. Agriculture Ecosystems & Environment 160: 66-74

Ravishankara AR, Daniel JS, Portmann RW (2009) Nitrous Oxide (N_2O): The Dominant Ozone-Depleting Substance Emitted in the 21st Century. Science 326(5949): 123-125

Rico C, Luis Rico J, Garcia H, Antonio Garcia P (2012) Solid - Liquid separation of dairy manure: Distribution of components and methane production. Biomass & Bioenergy 39: 370-377

Robertson GP, Vitousek PM (2009) Nitrogen in Agriculture: Balancing the Cost of an Essential Resource. In: Annual Review of Environment and Resources. p 97-125

Rochette P, Angers DA, Chantigny MH, Bertrand N, Cote D (2004) Carbon dioxide and nitrous oxide emissions following fall and spring applications of pig slurry to an agricultural soil. Soil Sci. Soc. Am. J. 68(4): 1410-1420

Rochette P, van Bochove E, Prevost D, Angers DA, Cote D, Bertrand N (2000) Soil carbon and nitrogen dynamics following application of pig slurry for the 19th consecutive year: II. Nitrous oxide fluxes and mineral nitrogen. Soil Sci. Soc. Am. J. 64(4): 1396-1403

Roelcke M, Li SX, Tian XH, Gao YJ, Richter J (2002) In situ comparisons of ammonia volatilization from N fertilizers in Chinese loess soils. Nutrient Cycling in Agroecosystems 62(1): 73-88

Rowell DM, Prescott CE, Preston CM (2001) Decomposition and nitrogen mineralization from biosolids and other organic materials: Relationship with initial chemistry. Journal of Environmental Quality 30(4): 1401-1410

Rubaek GH, Henriksen K, Petersen J, Rasmussen B, Sommer SG (1996) Effects of application technique and anaerobic digestion on gaseous nitrogen loss from animal slurry applied to ryegrass (Lolium perenne). Journal of Agricultural Science 126: 481-492

Ruser R, Flessa H, Schilling R, Beese F, Munch JC (2001) Effect of crop-specific field management and N fertilization on N_2O emissions from a fine-loamy soil. Nutrient Cycling in Agroecosystems 59(2): 177-191

Ruser R, Flessa H, Schilling R, Steindl H, Beese F (1998) Soil compaction and fertilization effects on nitrous oxide and methane fluxes in potato fields. Soil Sci. Soc. Am. J. 62(6): 1587-1595

Saenger A, Geisseler D, Ludwig B (2010) Effects of rainfall pattern on carbon and nitrogen dynamics in soil amended with biogas slurry and composted cattle manure. Journal of Plant Nutrition and Soil Science 173(5): 692-698

Saenger A, Geisseler D, Ludwig B (2011) Effects of moisture and temperature on greenhouse gas emissions and C and N leaching losses in soil treated with biogas slurry. Biol. Fertil. Soils 47(3): 249-259

Sahrawat KL (2008) Factors affecting nitrification in soils. Communications in Soil Science and Plant Analysis 39(9-10): 1436-1446

Sanchez M, Gomez X, Barriocanal G, Cuetos MJ, Moran A (2008) Assessment of the stability of livestock farm wastes treated by anaerobic digestion. International Biodeterioration & Biodegradation 62(4): 421-426

Scheftelowitz M (2013) Stromerzeugung aus Biomasse. 03MAP250, Zwischenbericht. In. DBFZ Deutsches Biomasseforschungszentrum, Leipzig, Germany.

Senbayram M, Chen R, Budai A, Bakken L, Dittert K (2012) N_2O emission and the $N_2O/(N_2O+N_2)$ product ratio of denitrification as controlled by available carbon substrates and nitrate concentrations. Agriculture Ecosystems & Environment 147: 4-12

Senbayram M, Chen R, Muehling KH, Dittert K (2009) Contribution of nitrification and denitrification to nitrous oxide emissions from soils after application of biogas waste and other fertilizers. Rapid Commun. Mass Spectrom. 23(16): 2489-2498

Sharpley AN, Chapra SC, Wedepohl R, Sims JT, Daniel TC, Reddy KR (1994) Managing Agricultural Phosphorous for Protection of Surface Waters - Issues and Options. Journal of Environmental Quality 23(3): 437-451

Sintermann J, Neftel A, Ammann C, Haeni C, Hensen A, Loubet B, Flechard CR (2012) Are ammonia emissions from field-applied slurry substantially over-estimated in European emission inventories? Biogeosciences 9(5): 1611-1632

Skiba U, Smith KA (2000) The control of nitrous oxide emissions from agricultural and natural soils. Chemosphere - Global Change Science 2: 379-386

Smith KA (1990) Greenhouse gas fluxes between land surfaces and the atmosphere. Progress in Physical Geography 14(3): 349-372

Smith KA, Jackson DR, Misselbrook TH, Pain BF, Johnson RA (2000) Reduction of ammonia emission by slurry application techniques. Journal of Agricultural Engineering Research 77(3): 277-287

Smith KA, McTaggart IP, Tsuruta H (1997) Emissions of N_2O and NO associated with nitrogen fertilization in intensive agriculture, and the potential for mitigation. Soil Use Manage. 13(4): 296-304

Smith P, Martino D, Cai Z, Gwary D, Janzen H, Kumar P, McCarl B, Ogle S, O'Mara F, Rice C, Scholes B, Sirotenko O (2007) Agriculture. In: Metz B, Davidson OR, Bosch PR, Dave R & Meyer LA (eds) Climate Change 2007: Mitigation. Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Sommer SG, Friis E, Bach A, Schjorring JK (1997) Ammonia volatilization from pig slurry applied with trail hoses or broadspread to winter wheat: Effects of crop developmental stage, microclimate, and leaf ammonia absorption. Journal of Environmental Quality 26(4): 1153-1160

Sommer SG, Genermont S, Cellier P, Hutchings NJ, Olesen JE, Morvan T (2003) Processes controlling ammonia emission from livestock slurry in the field. European Journal of Agronomy 19(4): 465-486

Sommer SG, Hansen MN, Sogaard HT (2004) Infiltration of slurry and ammonia volatilisation. Biosystems Engineering 88(3): 359-367

Sommer SG, Husted S (1995) The chemical buffer system in raw and digested animal slurry. Journal of Agricultural Science 124: 45-53

Sommer SG, Hutchings NJ (2001) Ammonia emission from field applied manure and its reduction - invited paper. European Journal of Agronomy 15(1): 1-15

Sommer SG, Jensen LS, Clausen SB, Sogaard HT (2006) Ammonia volatilization from surface-applied livestock slurry as affected by slurry composition and slurry infiltration depth. Journal of Agricultural Science 144: 229-235

Sommer SG, Olesen JE (1991) Effects of dry-matter content and temperature on ammonia loss from surface-applied cattle slurry. Journal of Environmental Quality 20(3): 679-683

Sommer SG, Olesen JE, Christensen BT (1991) Effects of temperature, wind-speed and air humidity on ammonia volatilization from surface applied cattle slurry. Journal of Agricultural Science 117: 91-100

Sommer SG, Sherlock RR, Khan RZ (1996) Nitrous oxide and methane emissions from pig slurry amended soils. Soil Biol. Biochem. 28(10-11): 1541-1544

Spott O, Russow R, Apelt B, Stange CF (2006) A N-15-aided artificial atmosphere gas flow technique for online determination of soil N_2 release using the zeolite Kostrolith SX6 (R). Rapid Commun. Mass Spectrom. 20(22): 3267-3274

Stehfest E, Bouwman L (2006) N_2O and NO emission from agricultural fields and soils under natural vegetation: summarizing available measurement data and modeling of global annual emissions. Nutrient Cycling in Agroecosystems 74(3): 207-228

Stevens RJ, Laughlin RJ (2001) Effect of liquid manure on the mole fraction of nitrous oxide evolved from soil containing nitrate. Chemosphere 42(2): 105-111

Stevens RJ, Laughlin RJ, Frost JP (1989) Effect of acidification with sulfuric-acid on the volatilization on ammonia from cow and pig slurries. Journal of Agricultural Science 113: 389-395

Stevens RJ, Laughlin RJ, Frost JP (1992) Effects of separation, dilution, washing and acidification on ammonia volatilization from surface-applied cattle slurry. Journal of Agricultural Science 119: 383-389

Stinner W, Rensberg N (2011) Perspektive der Biogasgewinnung aus nachwachsenden Rohstoffen - Bewertung von Substratalternativen zu Silomais (Studie). Leipzig

Subbarao GV, Ito O, Sahrawat KL, Berry WL, Nakahara K, Ishikawa T, Watanabe T, Suenaga K, Rondon M, Rao IM (2006) Scope and strategies for regulation of nitrification in agricultural systems-challenges and opportunities. Critical Reviews in Plant Sciences 25(4): 303-335

Sutton MA, Howard CM, Erisman JW, Billen G, Bleeker A, Grennfelt P, Grinsven Hv, Grizzetti B (2011) The European Nitrogen Assessment: sources, effects and policy perspectives Cambridge University Press, Cambridge

Svensson K, Odlare M, Pell M (2004) The fertilizing effect of compost and biogas residues from source separated household waste. Journal of Agricultural Science 142: 461-467

Tambone F, Genevini P, D'Imporzano G, Adani F (2009) Assessing amendment properties of digestate by studying the organic matter composition and the degree of biological stability during the anaerobic digestion of the organic fraction of MSW. Bioresour. Technol. 100(12): 3140-3142

Tambone F, Scaglia B, D'Imporzano G, Schievano A, Orzi V, Salati S, Adani F (2010) Assessing amendment and fertilizing properties of digestates from anaerobic digestion through a comparative study with digested sludge and compost. Chemosphere 81(5): 577-583

Terhoeven-Urselmans T, Scheller E, Raubuch M, Ludwig B, Joergensen RG (2009) CO_2 evolution and N mineralization after biogas slurry application in the field and its yield effects on spring barley. Applied Soil Ecology 42(3): 297-302

Thangarajan R, Bolan NS, Tian G, Naidu R, Kunhikrishnan A (2013) Role of organic amendment application on greenhouse gas emission from soil. Science of the Total Environment 465: 72-96

Thompson RB, Pain BF, Lockyer DR (1990) Ammonia volatilization from cattle slurry following surface application to grassland.1. Influence of mechanical separation, changes in chemical-composition during volatilization and the presence of the grass sward. Plant and Soil 125(1): 109-117

Thompson RB, Ryden JC, Lockyer DR (1987) Fate of nitrogen in cattle slurry following surface application or injection to grassland. Journal of Soil Science 38(4): 689-700

Thomsen IK, Olesen JE, Moller HB, Sorensen P, Christensen BT (2013) Carbon dynamics and retention in soil after anaerobic digestion of dairy cattle feed and faeces. Soil Biol. Biochem. 58: 82-87

Thomsen IK, Pedersen AR, Nyord T, Petersen SO (2010) Effects of slurry pre-treatment and application technique on short-term N_2O emissions as determined by a new non-linear approach. Agriculture Ecosystems & Environment 136(3-4): 227-235

Ussiri D, Lal R (2013) Soil Emission of Nitrous Oxide and its Mitigation. Springer, Dordrecht, The Netherlands

Vallejo A, Garcia-Torres L, Diez JA, Arce A, Lopez-Fernandez S (2005) Comparison of N losses (NO_3^-, N_2O, NO) from surface applied, injected or amended (DCD) pig slurry of an irrigated soil in a Mediterranean climate. Plant and Soil 272(1-2): 313-325

Vallejo A, Skiba UM, Garcia-Torres L, Arce A, Lopez-Fernandez S, Sanchez-Martin L (2006) Nitrogen oxides emission from soils bearing a potato crop as influenced by fertilization with treated pig slurries and composts. Soil Biol. Biochem. 38(9): 2782-2793

van Groenigen JW, Kasper GJ, Velthof GL, van den Pol-van Dasselaar A, Kuikman PJ (2004) Nitrous oxide emissions from silage maize fields under different mineral nitrogen fertilizer and slurry applications. Plant and Soil 263(1-2): 101-111

Vandermolen J, Vanfaassen HG, Leclerc MY, Vriesema R, Chardon WJ (1990) Ammonia volatilization from arable land after application of cattle slurry.1. Field estimates Netherlands Journal of Agricultural Science 38(2): 145-158

VanderZaag AC, Jayasundara S, Wagner-Riddle C (2011) Strategies to mitigate nitrous oxide emissions from land applied manure. Animal Feed Science and Technology 166-67: 464-479

Vandre R, Kaupenjohann M (1998) In situ measurement of ammonia emissions from organic fertilizers in plot experiments. Soil Sci. Soc. Am. J. 62(2): 467-473

Velthof GL, Kuikman PJ, Oenema O (2003) Nitrous oxide emission from animal manures applied to soil under controlled conditions. Biol. Fertil. Soils 37(4): 221-230

Velthof GL, Mosquera J (2011) The impact of slurry application technique on nitrous oxide emission from agricultural soils. Agriculture Ecosystems & Environment 140(1-2): 298-308

Velthof GL, Oenema O, Postma R, VanBeusichem ML (1997) Effects of type and amount of applied nitrogen fertilizer on nitrous oxide fluxes from intensively managed grassland. Nutrient Cycling in Agroecosystems 46(3): 257-267

Webb J, Menzi H, Pain BF, Misselbrook TH, Dammgen U, Hendriks H, Dohler H (2005) Managing ammonia emissions from livestock production in Europe. Environmental Pollution 135(3): 399-406

Webb J, Pain B, Bittman S, Morgan J (2010) The impacts of manure application methods on emissions of ammonia, nitrous oxide and on crop response-A review. Agriculture Ecosystems & Environment 137(1-2): 39-46

Weier KL, Doran JW, Power JF, Walters DT (1993) Denitrification and the dinitrogen nitrous-oxide ratio as affected by soil-water, available carbon, and nitrate. Soil Sci. Soc. Am. J. 57(1): 66-72

Weiland P (2010) Biogas production: current state and perspectives. Applied Microbiology and Biotechnology 85(4): 849-860

Well R, Butterbach-Bahl K (2010) Indirect Emissions of Nitrous Oxide from Nitrogen Deposition and Leaching of Agricultural Nitrogen. In: Smith K (ed) Nitrous Oxide and Climate Change. Earthscan Ltd., London. p 232

Well R, Kurganova I, de Gerenyu VL, Flessa H (2006) Isotopomer signatures of soil-emitted N_2O under different moisture conditions - A microcosm study with arable loess soil. Soil Biol. Biochem. 38(9): 2923-2933

Wrage N, Velthof GL, van Beusichem ML, Oenema O (2001) Role of nitrifier denitrification in the production of nitrous oxide. Soil Biol. Biochem. 33(12-13): 1723-1732

Wulf S, Maeting M, Bergmann S, Clemens J (2001) Simultaneous measurement of NH_3, N_2O and CH_4 to assess efficiency of trace gas emission abatement after slurry application. Phyton-Annales Rei Botanicae 41(3): 131-142

Wulf S, Maeting M, Clemens J (2002a) Application technique and slurry co-fermentation effects on ammonia, nitrous oxide, and methane emissions after spreading: I. Ammonia volatilization. Journal of Environmental Quality 31(6): 1789-1794

Wulf S, Maeting M, Clemens J (2002b) Application technique and slurry co-fermentation effects on ammonia, nitrous oxide, and methane emissions after spreading: II. Greenhouse gas emissions. Journal of Environmental Quality 31(6): 1795-1801

Zaman M, Saggar S, Blennerhassett JD, Singh J (2009) Effect of urease and nitrification inhibitors on N transformation, gaseous emissions of ammonia and nitrous oxide, pasture yield and N uptake in grazed pasture system. Soil Biol. Biochem. 41(6): 1270-1280

Zhang RH, Westerman PW (1997) Solid-liquid separation of animal manure for odor control and nutrient management. Applied Engineering in Agriculture 13(5): 657-664

Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM (2009) Mixed Effect Models and Extensions in Ecology with R. Springer, New York

7 Curriculum Vitae

Personal data

Name	Ulrike Kerstin Wolf
Date and place of birth	19th October 1984 in Bayreuth (Germany)
Nationality	German

Education

2010 – 2014	Doctoral candidate, Institute of Geoecology, Technical University of Braunschweig
2010 – 2012	Scholarship Student in the DFG Research Training Group 1397 "Regulation of soil organic matter and nutrient turnover in organic agriculture", University of Kassel / Witzenhausen
2006 – 2009	Studies of Geography (diploma), Georg-August-University of Göttingen
2004 – 2006	Studies of Geography, University of Regensburg
2003	Abitur, Wirtschaftswissenschaftliches und mathematisch-naturwissenschaftliches Gymnasium, Bayreuth

Publications

Chapter 3 of this thesis has been published in Nutrient Cycling in Agroecosystems as follows:

U. Wolf, R. Fuß, F. Höppner, H. Flessa (2014):
„Contribution of N_2O and NH_3 to total greenhouse gas emission from fertilization – results from a sandy soil fertilized with nitrate and biogas digestate with and without nitrification inhibitor"
Nutrient Cycling in Agroecoystems (2014) 100:121–134
DOI 10.1007/s10705-014-9631-z

Danksagung

Das Promotionsprojekt wurde im Rahmen des DFG Graduiertenkollegs 1397 „Steuerung von Humus- und Nährstoffhaushalt in der ökologischen Landwirtschaft“ angefertigt und von der Deutschen Forschungsgemeinschaft finanziell unterstützt.

Als erstes möchte ich mich für die Anregung und Konzeption der vorliegenden Arbeit ganz herzlich bei Prof. Dr. Heinz Flessa bedanken. Ihm gebührt auch besonderer Dank für die intensive Betreuung und für viele konstruktive Diskussionen, die mir bei der Erstellung dieser Arbeit sehr geholfen haben.

Bei Herrn Prof. Dr. Rolf Nieder bedanke ich mich für die Übernahme des Erstgutachtens, seine hilfreichen Ratschläge und für sein Engagement im Promotionsgremium.

Für die vielfältige Unterstützung bei der Planung, Durchführung und methodischen Auswertung des Laborversuchs bedanke ich mich ganz herzlich bei Dr. Reinhard Well und Dr. Mirjam Helfrich.
Besonders möchte ich mich außerdem bei Dr. Roland Fuß bedanken, der jederzeit ein verlässlicher Ansprechpartner bei allen Fragen der statistischen Auswertung war.
Außerdem danke ich Dr. Annette Freibauer für ihre Unterstützung bei der Planung des Feldversuches in Italien und ihre engagierte Mithilfe im Gelände.

Mein Dank gilt den italienischen Kollegen am CRPA in Reggio Emilia, insbesondere Paolo Mantovi und Laura Valli für die sehr gute Zusammenarbeit sowie Fabrizio Ruozzi für die Bereitstellung der Feldflächen, die verlässliche Hilfe bei allen Fragen rund um die Umsetzung im Gelände und die herzliche Aufnahme auf seinem Hof. Großer Dank gebührt Loris Manicardi, ohne dessen Einsatz die Geländearbeit nicht machbar gewesen wäre.

Ich danke Dr. Frank Höppner und seinen Mitarbeitern vom Julius Kühn Institut für Pflanzenbau und Bodenkunde in Braunschweig für die sehr gute Zusammenarbeit bei dem Feldversuch. Insbesondere danke ich Herrn Dirk Hillegeist für die spontane und unerschöpfliche Mitarbeit bei scheinbar endlosen Gärversuchen.

Wesentlich zum Gelingen dieser Arbeit hat auch die tolle Arbeitsatmosphäre am Institut für Agrarklimaschutz in Braunschweig beigetragen, dafür danke ich allen Kolleginnen und Kollegen. Ein großes Dankeschön gilt dem Zentral- und dem Isotopenlabor für die zügige Messung zahlreicher Proben sowie dem technischen Personal für die Unterstützung im Labor und Feld!

Ich danke außerdem den Hiwis, die während der Feld- und Laborversuche unermüdlich und mit sehr großem Einsatz gearbeitet haben.

Meinen Mitdoktorandinnen im Haupthaus, Greta Roth, Jasmin Miltz, Lena Rohe, Marianna Deppe und Nina Eibisch danke ich für das freundschaftliche und entspannte Arbeitsklima, zahllose fachnahe und fachfremde Gespräche, Motivation und die Bereicherung des Arbeitsalltags. Danke, dass ich mich auch sehr spontan auf eure Hilfe im Feld oder Labor verlassen konnte!

Meinen Eltern möchte ich herzlich dafür danken, dass sie immer voll hinter mir standen und mich unterstützt haben! Meinem Freund Hermann Achenbach gebührt größter Dank für seine endlose Geduld und starken Nerven sowie die vielfältige Hilfe im Feld und im Labor, ohne die diese Arbeit nicht möglich gewesen wäre!